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THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

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Physiological and Morphological Correlations in Herbaceous Angiosperms
E. C. Jeffrey and R. E. Torrey 1
(With Plates I–VII and four figures)

After-ripening and Germination of Juniperus Seeds. Contributions from the Hull Botanical Laboratory 275 - - - - - Dean A. Pack 32
(With one figure)

Gametophyte and Sex Organs of Reboulia hemisphaerica. Contributions from the Hull Botanical Laboratory 276 - - - Arthur W. Haupt 61
(With twenty-one figures)

Current Literature

Notes for Students - - - - - - - - - - - 75

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EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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VOLUME LXXI NUMBER 1

THE
BOTANICAL GAZETTE

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PHYSIOLOGICAL AND MORPHOLOGICAL CORRELATIONS IN HERBACEOUS ANGIOSPERMS[1]

E. C. JEFFREY AND R. E. TORREY

(WITH PLATES I–VII AND FOUR FIGURES)

The general significance of the herbaceous type seems to have first been clearly realized in the classification of plants put forward by CLUSIUS in the seventeenth century. This author divided plants into large groups based on their habit, much in the same manner that the earlier zoological classifications summarized animals under such groups as flying, creeping, swimming, etc. The idea, however, has long been abandoned that the habit of life can serve as a satisfactory basis for either systematic or phylogenetic classification. This consideration does not in any way eliminate the herbaceous type as a physiological assemblage of the greatest importance in the present epoch of the history of the earth. In this connection the question arises as to the origin of a physiological group which is of such high significance. The older view implicitly, if not in actual statement, assumed for the herbaceous type a position of primitiveness. This attitude is well illustrated by such classics as GRAY'S *Structural Botany* and SACHS'S *Lehrbuch*. In these works the woody stem is represented diagrammatically as originating from the linking up of originally

[1] Contribution from the Laboratories of Plant Morphology of Harvard University.

separate fibrovascular bundles by a so-called interfascicular cambium. The parts of the woody cylinder corresponding to the supposed primitive bundles are known in this connection as fascicular wood, and the sometimes depressed segments which intervene as interfascicular wood. When the sunken segments occur (comparatively rarely, and not widely enough distributed to warrant a hypothesis of the origin of the woody stem in general), the old view regarded their sunken topography as resulting from their later origin through the activity of the belated so-called interfascicular cambium. As has been shown by previous reports from this laboratory, the depression of segments of the stem in trees, vines, and woody herbs is a question of growth mechanics and has nothing to do with belated cambial activity. It is intimately connected, in fact, with the appearance of approximated pairs of large rays, resulting from a process of aggregation of the smaller rays of the general wood in proximity to the entering leaf traces. The large rays undoubtedly make their appearance to supply the greater storage necessities, which are a special feature of the more efficient representatives of the dicotyledonous Angiosperms. In other words, the large rays so universally characteristic of the thicker and more woody regions of herbs, whether aerial or terrestrial, are clearly a physiological response to the climatic conditions of modern times in cooler latitudes and higher altitudes, where storage either in stem or seeds becomes of paramount importance.

Since it obviously can no longer be maintained that the woody stem is derived from the apparently simpler herbaceous axis, the question naturally arises whether the reverse relation of origin exists between the two types. To those possessing a knowledge of the past of the great groups of vascular plants this possibility presents no difficulties whatever, since it is certainly known that many of the herbaceous living representatives of cryptogamic groups have had as ancestors forms of strikingly arboreal habit. This situation indeed provoked a long continued contest as to whether the treelike Cryptogams of the paleozoic periods were not in reality seed plants, as are arboreal types with secondary growth at the present day. It has been emphasized

by one of us (4), however, that the herbaceous Cryptogams are distinctly the result of degenerative changes in the older and ancestral forms. The herbaceous type in the Angiosperms, as will be pointed out in the sequel, has had an entirely different mode of origin from that illustrated by many existing herbaceous Cryptogams.

Nearly a decade ago EAMES (2), in a publication from this laboratory, made a comparative study of the herbaceous type, which was particularly focussed on the Rosaceae, since this group presents in relative abundance closely related forms of woody and herbaceous texture. This investigator concluded that there is clear evidence in the Rosaceae for the origin of herbaceous stems from woody, as a consequence of the formation of large storage rays in relation to the incoming foliar traces. These rays usually extend some distance below the foliar trace, and also may be developed above it. In the horizontal aspect of the axis, in the region of the node of stems with a well developed fibrovascular cylinder, the storage parenchyma related to the leaf traces can be seen subtending them externally. The development of these masses of storage tissues in woody stems and in correlation to the vascular supply of the leaves (since the masses in question not only subtend the foliar traces vertically but, in cylinders of any thickness, likewise in the radial dimension as well) automatically results in the transformation of the continuous dicotyledonous woody cylinder (ultimately at any rate) into a circular series of discontinuous fibrovascular segments, the fibrovascular bundles so characteristic of herbaceous stems. Accompanying the development of the large foliar rays, as pointed out by EAMES, is the final degeneracy of the rays of moderate size, which are normally characteristic of the woody cylinder of arboreal Angiosperms.

In an article published a few years later, SINNOTT and BAILEY (5) attack EAMES's conclusions, and while admitting the derivation of herbaceous forms from arboreal or woody, they question the existence of foliar rays subtending the foliar traces vertically and horizontally. They admit the frequent presence of foliar rays in the subterranean stem of herbs, but deny that this has any bearing on the question of the origin of the herbaceous type. They

consider that the presence of foliar rays in aerial stems is rare, and that this supposed absence constitutes a weak point of the hypothesis of the derivation of the herbaceous type, which they ascribe to the senior author of the present article. In a recent article WHITAKER (4) has demonstrated clearly the insecure basis of the assertion of SINNOTT and BAILEY as to the absence of foliar rays in the aerial axis of the Compositae, perhaps the most important and certainly the most easily available group which is represented in temperate climates by typical herbs. One of us (4) has likewise recently discussed the anatomy of the flowering leafy stem of *Helianthus*, with similar conclusions.

It will serve a useful purpose to begin the description of the herbaceous stem, from the aspects included in the present discussion, with a description of the aerial stem in certain common Compositae. Fig. 1 shows a transverse section of the stem of *Aster novae-angliae*, common in the eastern states. The lower region of the axis above ground is represented. To the left at the top of the figure can be seen a marked depression of the surface of the stem. This corresponds to the median foliar ray of a leaf attached to the stem slightly above the plane of section. This ray is characterized not only by the depression it causes on the periphery of the stem, but also by the absence of vessels in its external region. Below this ray and on the left occurs another foliar ray, corresponding to one of the two lateral traces of the leaf under consideration. This ray is substantially like the one subtending the median trace, but is smaller in size and causes a less marked depression on the surface of the woody cylinder and on the periphery. The foliar ray of the right-hand lateral trace is about as far to the right of the median ray as the left ray is on the opposite side. It will be noted that the depression in this case is very slight, and that the foliar ray is deeper radially and less broad than the other two similar structures. Since the traces do not always penetrate the stem at the same level, the rays which correspond to the three traces of the same leaf usually do not present the same appearance in details in a given section. An examination of the rest of the woody cylinder in fig. 1 shows that no foliar rays closely resembling the three under discussion can be distinguished. This results from the fact that

the foliar rays of the species of *Aster* under consideration extend only a slight distance downward in their normal form, and as a consequence in the lower and stouter region of the part of the stem above ground usually only three typical leaf rays can be seen in a given plane of section.

Fig. 2 shows a transverse view of the same *Aster* in the upper region of the aerial stem. Here the woody cylinder appears much thinner, in spite of the fact that the magnification is considerably higher than in fig. 1. A projection on the upper surface marks the position of the median trace of a leaf belonging to the nodal region from which the section has been made. The higher part of the stem, as is often the case in above ground herbaceous axes, shows the leaf traces and their corresponding foliar rays as projections from the surface of the woody cylinder, and not depressed, as is the normal condition for these structures in herbs of woody texture or in the woody lower region of the aerial stem of more typically herbaceous axes. In addition to the three projecting leaf traces and their corresponding foliar rays, which occupy adjacent positions on the upper surface of the woody cylinder in fig. 2, are to be seen 5 or 6 less prominent traces and corresponding foliar rays belonging to leaves higher up on the stem. In figs. 3–5 is reproduced the whole of the woody cylinder of fig. 2, on a somewhat higher scale of magnification. It will be seen from figs. 2–5 that there are 8 leaf traces clearly obvious, alternating with as many "common" or "cauline" segments. This interesting situation results from the fact that in the upper more herbaceous region of the stem the foliar rays extend, as such, much deeper down than in the case of the lower woody region of the same aerial axis. Consequently a number of leaf rays corresponding to the traces of leaves at several nodes can be distinguished in a single transverse section through the higher region of the stem. SINNOTT and BAILEY (5) have denied the possibility of the conditions represented in figs. 2–5. The topography in question is in fact extremely common in herbs of a certain degree of advance from the woody primitive ancestral forms. Naturally it does not occur in extremely woody herbs, on the one hand, because in these very few foliar rays can usually be seen at one time, and these in rather close vertical

proximity to the next superior node. In extremely slender herbaceous stems, on the other hand, by the thinning of the woody cylinder, the foliar ray radially external to the leaf trace is eliminated, and only the flanking portions of the ray on either side of the foliar trace in its upward vertical course in the stem can be distinguished. It follows that an alternation of foliar rays and stem bundles is extremely common in herbs which are transitional in their texture, but naturally does not occur in either extremely woody herbs or in those which are strikingly herbaceous.

In fig. 6 is shown the foliar ray and its inwardly subtending leaf trace. The foliar trace may be recognized as radially disposed groups of vessels separated by equally radial bands of parenchyma. Externally the vessels give place to fibers and parenchyma, which constitute the external or confronting portion of the foliar ray. Lateral to the vascular leaf trace on either side can be distinguished the flanking portions of the foliar ray. It follows that a foliar ray may consist of parts flanking the leaf trace and separating it from the adjacent stem segments, as well as a region subtending the foliar strand externally. In thinner stems the latter portion progressively disappears, until only the flanking portions of the ray persist. This situation has recently been represented in diagram by WHITAKER. SINNOTT and BAILEY (6) have, however, quite failed to understand the situation. Since the genus *Aster* is a somewhat woody herb the topography represented in figs. 2–5 is usual in the upper region of the stem of various species.

We may now pass to a more herbaceous illustration of the Compositae, namely *Helianthus*, which has already been used (4) to exemplify the anatomical conditions in regard to the topography of the foliar rays. Corresponding to its less woody structure is the fact that foliar rays possessing depth enough to subtend as well as flank the leaf trace are confined usually to the lower portions of the aerial stem. In the superior portion of the latter the leaf rays are purely flanking in their development, on account of the slender nature of the woody cylinder. Disregarding the latter condition for the moment, we may turn our attention to the lower region of the subaerial stem. Fig. 7 is a foliar ray from the base of the stem of *Helianthus annuus*. Obviously the continuity of the woody cylinder is broken by a strongly developed radial band

of storage parenchyma, the foliar ray subtending the leaf trace in its vertical course in the stem. Along the inner margin of the ray may be seen the elements constituting the foliar trace. Fig. 8 represents the foliar ray of *Helianthus orgyalis*, a perennial and somewhat more woody species than *H. annuus*. This is in accordance with a general principle, for, other things being equal, the more woody an herb is, the longer radial and the less tangential and vertical extension have its foliar rays. Conversely, the more herbaceous the texture, the shorter the radial extension of the leaf rays and the greater their vertical length. Another feature of contrast can be distinguished between the rays shown in figs. 7 and 8. In the former the ray is quite homogeneous in structure, while in the latter narrower fibrous elements constitute radial bands in the substance of the ray. This contrast is a common one between the foliar rays of woody and more advanced herbs.

We may now turn to the relative size of the foliar traces and rays in more and less herbaceous forms, as well as the contrasts in the structure of the rays presented in these various modifications of the herbaceous type. The best way to make these important features clear is to choose corresponding regions of the stem, progressively more herbaceous in their texture, from different species of the same genus. Fig. 10 shows the vertical tangential view of a foliar ray from the lowest region of the aerial stem of the somewhat woody *Helianthus hirsutus*. It will be observed that the structure of the foliar ray in this instance is far from homogeneous, since bands of fibers are common in its substance, and with them vessels likewise occur. In this instance the foliar ray is obviously in the process of organization from the ordinary wood, namely, from smaller rays, separated by strands of vessels and fibers. In more advanced herbs belonging to *Helianthus*, as will shortly appear, the fibers and vessels progressively disappear, until the final result is a large mass of radial and longitudinal storage tissue, the typical foliar ray.

Fig. 10 represents the foliar ray in a condition of aggregation from smaller rays. SINNOTT and BAILEY (**6**) have denied the possibility of the appearance of foliar rays as the result of aggregation. Their error in this respect has been pointed out recently by HOAR (**3**), and consequently need not be referred to further here.

In one of the uppermost members of the aggregation (fig. 10) may be seen a darker dot, representing the transverse section of the outgoing foliar trace. The trace is obviously extremely small in size. Fig. 14 illustrates a portion of fig. 10 under a higher degree of magnification. The composite character of the foliar ray is now more easily seen, for rays, fibers, and vessels are all evident as component parts of the aggregate foliar ray. Fig. 11 is a tangential view of the foliar ray of *H. tuberosus*, on the same scale of magnification as fig. 10, representing a similar aspect of the ray in *H. hirsutus*. The contrasts between the two figures are both interesting and important from the standpoint of the question of the origin of the herbaceous type. The leaf ray in fig. 11 is much more clearly developed than in fig. 10. The fibers are much reduced in quantity and very few vessels are to be seen. Another important contrast is presented both by the size of the foliar ray and of the foliar trace to which it is related. The leaf strand appears as a dark, somewhat triangular spot in the upper part of the ray, as shown in fig. 11. In fig. 9 is shown a portion of the ray of *H. tuberosus*, under somewhat higher magnification than fig. 11. By comparing figs. 9 and 14, which represent details of the foliar rays of *H. tuberosus* and *H. hirsutus* under the same degree of magnification, it is clear that the ray in the former and more herbaceous species is much larger, and also contains more parenchymatous storage tissue than in the latter and more woody species.

The greatest contrast in every detail of organization, as well as in relative size, however, is presented by *H. annuus*, the most herbaceous of the three compared species. In fig. 12 is shown the tangential view of the leaf ray of this sunflower, under the same magnification as in figs. 10 and 11. The huge size of the ray is clearly seen. Not less striking is its homogeneous organization, resulting from the virtual disappearance of the fibers and vessels, which betray the aggregated character of the rays in *H. hirsutus* and *H. tuberosus*. The foliar ray in *H. annuus* is of the type which we have called compound, since with a homogeneous structure it still betrays evidence (from the comparative anatomical standpoint) of having been organized by the fusion of a number of ordinary wood rays in the vicinity of the foliar trace, with the

concurrent transformation of their separating fibers and vessels into storage elements. This results in the final formation of a large homogeneous complex of radial storage tissue, intimately connected both topographically and physiologically with the leaf trace. Fig. 12 shows also the leaf trace enlarged to the same proportionate size as the huge storage ray to which it belongs.

It is clear from the comparison of the foliar rays in the species of sunflower that the size of the ray, the size of the leaf trace, and the degree of parenchymatous homogeneity of the foliar ray, all directly correspond to the degree of herbaceous development of the species. *Helianthus annuus*, which is not only the most herbaceous of the three, but is of such vigor as to be able to proceed from seed to seed in a single season, is characterized by marked superiority in all three particulars. Fig. 13, showing the structure of the leaf ray of *H. annuus* under the same enhanced magnification as the other two species in figs. 9 and 14, makes it clear that the structure of the foliar ray in the former species is characterized by enough variety in the dimensions of its constituent elements to reveal its composite derivation from the modification of wood rays, fibers, and vessels.

Having made a comparative study of the foliar ray in progressively more herbaceous species of *Helianthus* in the lower region of the aerial stem, attention may be given now to the considerations represented in the upper nodes of the axis. Fig. 15 illustrates the outstanding leaf trace of an upper node of the stem of *H. tuberosus*. It is separated from the stem bundles on either side by flanking parenchymatous bands of the foliar ray. The large radial extension of the foliar ray which marks its organization in the lowest part of the stem has become progressively reduced in the region of higher nodes, until at the level of fig. 15 it has entirely disappeared. This simple geometrical condition has been diagrammed recently by WHITAKER (7) and need not be further considered here.

Another feature of interest, which is particularly obvious in the upper nodes, is the condition of cambial activity in the foliar and cauline bundles respectively. Fig. 16 represents the stem or "common" bundle immediately to the right of the foliar trace in fig. 15. Above is to be seen the fibrous pericycle of the bundle

protecting the phloem. Separating the phloem from the xylem is a well marked cambial region, characterized by the regularly seriate arrangement of its cells. Fig. 17 represents the foliar trace of fig. 15, under the same magnification as fig. 16. It is clear that the cambial activity found in the stem bundle is conspicuous by its absence in the leaf trace, since there are no regularly radially arranged cells intervening between xylem and phloem.

Before discussing the significance from the evolutionary standpoint of the facts elucidated in figs. 15–17, it will be well to consider the conditions present in the bundles of the root, using *Aster*, since this genus presents a more illuminating range of organization in the root than that which characterizes *Helianthus*. Fig. 18 represents a transverse section of the mature root of *Aster Shortii*. Root hairs are conspicuous by their absence, and the strong development of the secondary wood is very obvious. In the midst of the secondary organization of the wood can be seen a small 5-angled star of primary xylem, the points of which alternate with the 5 dark hued masses of phloem seen on the margin of the fibrovascular cylinder of the root. Fig. 19 is a transverse section of a young root of the same species of *Aster*, in which the secondary growth of the xylem is just beginning. The primary xylem star with its 5 points alternating with 5 angles of phloem can distinctly be seen. Root hairs are present on the surface of the root, but are beginning to disappear, as is normally the case in roots in which secondary structures have begun to form. Fig. 20 represents a very different type of root, in which the organs are distinguished by the indefinite persistence of the root hairs. This condition is well shown by the varieties of the somewhat inconstant species *Aster umbellatus*. Here the roots continue as hirsute structures for several years, and in fact this condition persists until they have become quite dark with age and have begun to decay. Fig. 20, which represents an old root of this species, shows that the persistence of the root hairs is paralleled by the absence of secondary structures in the cylinder of the organ. This is an interesting general condition of the organization of permanently hairy, and, as a consequence, presumably persistently absorbent roots.

Fig. 21 shows the very herbaceous stem of *Aster tataricus*, which differs from the American species in the strikingly soft texture and great vigor and thickness of its stem. This condition is shown in the transverse section by the large pith surrounded by a series of a distinctly separated and slender fibrovascular bundles. Another distinguishing feature is the large number of foliar traces which proceed from each leaf, these being as many as 7 (as may be seen in the upper region of the figure) in contrast with the 3 which mark the numerous woody species of *Aster*. It is clear, therefore, that *A. tataricus* is a particularly well developed herb, and in this respect presents a marked contrast with the predominantly woody herbaceous species of *Aster*. The roots of *A. tataricus* are like those of *A. umbellatus* in that they are permanently without development of the secondary wood, and likewise have persistent root hairs, albeit these are much more delicate than in the latter species.

Bringing together these results, we conclude that in woody species of *Aster* persistent root hairs and absence of secondary wood are correlated features of the organization of the root. In roots with evanescent root hairs the disappearance of these marks the beginning of the secondary activities in the wood. In *Aster tataricus*, which is remarkably herbaceous in its general habit in contrast with the species of the genus as a whole, a similar correlation is found between the persistence of root hairs and the absence of secondary growth. This will not be discussed further at the present time, but it will elsewhere be shown that there is a general correlation between a high absorptive capacity of the root (associated morphologically with persistent root hairs) and the absence of secondary woody developments in the central cylinder of the root. In other words, the herbaceous habit in roots is marked both by high absorptive efficiency and by the absence of secondary woody growth.

In the light of these preliminary results in the case of the root, the situation already described for the leaf traces of *Helianthus* becomes particularly significant. A general examination of the species of this genus, particularly of those presenting in a more marked degree the herbaceous habit, indicates that cambial activity

is very generally absent in the strands which conduct the assimilates from the leaf into the stem. The conducting efficiency of the foliar trace would obviously be considerably reduced were part of the assimilates to be used in the growth of the bundles of the foliar traces instead of being transferred intact to storage in the stem or seeds. High conductive efficiency, notably correlated in herbs with the assimilative productiveness of the leaf, as will be pointed out later, is in general associated with the loss of cambial activity in the foliar traces as they enter the cylinder of the stem. In the root the most obvious interpretation of the absence of secondary woody growth is the unfavorable effect that a jacket of secondary elements would have on the ease of penetration of the water absorbed by the root hairs into the water-conducting tissues of the central cylinder. Since the essential conditions of the loss of a cambium are different in stem and root, it is clear that both may not present the feature of the loss of secondary activities in their fibrovascular structures at the same time. In other words, the stem may be herbaceous and the root woody; or vice versa, the stem may be woody and the root herbaceous. The former condition is well exemplified by certain species of *Papaver*, while *Aster* furnishes examples of the latter.

We may now turn to a type which is still more delicate in its herbaceous texture than are either *Aster* or *Helianthus*. Like the sunflower, the buttercup often serves as an example of the herbaceous type in laboratory exercises, and for that reason it is chosen in this connection, as illustrating a common and easily obtained plant. Fig. 22 illustrates the organization of a small stem of *Ranunculus acris* in the region of the branching node. The larger cylinder below represents that of the main axis, while the branch cylinder is smaller and appears above. Inclosing the branch and fused with it is the base of a leaf, in which may be distinguished 5 fibrovascular bundles, the leaf traces. As the cylinders of axis and branch come together, the phloem of their uppermost and lowest bundles respectively face one another. In fig. 23 is shown a somewhat lower plane of section in a rather larger stem than that illustrated in fig. 22. Here the union of secondary and main axes has taken place with interesting results. In the

outer region of the pith corresponding to the medulla of the secondary axis are several amphivasal concentric fibrovascular bundles, precisely similar to those so commonly found in the stem of monocotyledons. The amphivasal strands are the result of the fusion of the confronting bundles of main axis and lateral branch by their phloems. This condition is not an uncommon one for dicotyledonous stems of marked herbaceous nature, particularly when they bear large leaves with many foliar traces entering the axis at the nodes. Other examples of this feature will be supplied later.

Fig. 24 shows a section of the stem of a buttercup at some distance below the node. A rather old stem has purposely been chosen, so that the identity of the foliar traces in the stem may readily be distinguished. In such stems, in the later summer, gummosis invades the cavities of the vessels of the foliar traces, a feature which makes the leaf traces stand out even in low magnifications. To the extreme right is seen the median trace of the leaf, and above and below on either hand are the lateral traces, two on either side. The foliar traces are emphasized by their blackness, resulting from the mucilaginous contents of their vessels. Fig. 25 is a portion of a section similar to that illustrated in fig. 24, somewhat more highly magnified. It is now quite easy to discern that the two outside bundles have vessels plugged with darkened contents, while the vascular structures in the central bundles appear quite clear and devoid of gummosis. Fig. 26 furnishes a further example of the phenomenon of gummosis in the external bundles, which here as in the other instances are foliar traces. The leaf trace to the right is much smaller than its counterpart on the left, and represents in fact an extreme lateral trace of the leaf.

In fig. 27 appears a part of a stem preserved in the early summer, in which as a consequence gummosis is not present in the vessels of the foliar traces. The magnification is greater than in the two preceding figures, and more details of the structure of the fibrovascular bundles are evident. Obviously the two larger bundles at the ends present a different structure from those toward the center. This expresses itself in a more parenchymatous organization of the xylem, in which comparatively few vessels are distributed

in a large amount of heavily pitted wood parenchyma. In the cauline strands, on the contrary, the xylem is more vascular and the parenchymatous elements are less abundant and thinner-walled. A further distinction is the absence of cambial activity in the foliar traces, a situation which leads to a less regular arrangement of the cells than is found in the central strands. In fig. 29 appears another illustration on a somewhat higher scale of magnification than that in fig. 27. The bundle to the right, which is a cauline strand, shows distinct evidence of cambial activity such as is figured in STRASBURGER'S *Botanische Practicum*. On the other hand, the foliar strand, which here lies to the left, is without cambial activity, as indicated by radial regularity. Fig. 31 is a partial view of another transverse section, in which a large cauline bundle lies on the left, while on the right is another foliar bundle, in this case also distinguished by the absence of cambial activity. To make clear that cambial activity is really absent in the foliar strands of the buttercup in the upper part of their course in the cylinder of the axis, several figures under considerable magnification have been introduced. Fig. 28 shows a foliar strand under a moderately high magnification. It is clear that cambial activity is absent here, since there are no regularly radially arranged rows of cells intervening between xylem and phloem as is the case with the stem bundles. Moreover, the less woody organization of the xylem is a further consequence of this absence of cambial increments. Fig. 30 shows another foliar bundle in the stem under somewhat higher magnification. The absence of cambial growth is here still clearer. Fig. 32 shows a still more enlarged view of one of the leaf traces in the stem, and the absence of any evidence of secondary activity is apparent.

It is obvious that in the buttercup, precisely as in the sunflower and to a less degree in the aster, there is a distinct and striking intermission of cambial activity in those fibrovascular strands in the stem which have entered from the leaves. In the case of *Aster* attention has been called to the absence of secondary additions of the xylem in the case of species in which the root hairs are indefinitely persistent. The root of the buttercup, as has long been known, is without secondary accretions to the wood. Fig. 33

shows the root of *Ranunculus acris*. The absence of secondary activities is striking. Sections through the subterranean perennial stem of the buttercup show leaf traces and root bundles in the axial region, both contrasting with the bundles of the stem proper by the entire absence of cambial activity. It is clear that in the buttercup the herbaceous habit has completely taken possession of both stem and root, so that secondary activities are equally conspicuous by their absence, both in strands destined to the leaves and to the roots. Even in the perennial and often rather thickened subterranean stem of *R. acris*, the fibrovascular tissues do not ordinarily show themselves so well developed as to manifest the presence of typical foliar rays subtending the foliar traces. One can easily find such rays in the perennial subterranean stem of *Thalictrum*, however, and also in the aerial stem of various woody species of *Clematis*. It is obvious that typical foliar rays associated with distinctly woody perennial stems lie in the phylogenetic past of extremely herbaceous representatives of the Ranunculaceae, precisely as they do in the case of the more woody herbaceous types which ordinarily and likewise represent the Compositae in temperate climates.

We may now pass to other illustrations of the herbaceous type as characterized by delicate stems and numerous leaf traces entering the stem at the node. The Umbelliferae will serve as our first illustration in this connection. Fig. 34 is a total transverse section of a node in the aerial axis of a species of *Sanicula*. Uppermost and toward the right is a leaf base in the act of fusing with the stem. It shows the presence of a considerable number of foliar traces, which become still more numerous a little lower down by subdivisions of the original strands. Axillary to the foliar base is the cylinder of a lateral branch. This spreads out laterally over the surface of the main cylinder, in such a manner that the bundles of the branch present themselves to those of the main axis by their phloems. Fusion of these mutually confronting strands takes place in such a manner that a number of amphivasal strands results. The formation of these begins in the axillary region and proceeds progressively downward on either side. In fig. 35 appears a section somewhat lower down on the same axis as that shown

in fig. 34. The foliar traces are more numerous at this level, and are beginning to draw in toward the fibrovascular cylinder of the main axis. It will be seen that the bundles of the axis are amphivasal in the upper middle region. On the left is one of the bundles of the branch pursuing a tangential course to become fused with the face of the more lateral bundles of the cylinder of the main axis. The bundles of the main stem become amphivasal for nearly two-thirds of the perimeter of the cylinder before the union of the branch with the parent axis is completed. One of the amphivasal strands from the median upper region of fusion is shown in fig. 36. It is clear that the organization is quite similar to that of the Monocotyledons, the only contrast being in the presence of secondary growth. This, however, is not a matter of great importance from the evolutionary standpoint, since often in the Monocotyledons secondary activities can be recognized in the nodal region of the stem, as has been pointed out by CHRYSLER (1) and others. It is of interest to observe that in this genus the fusions which result in the formation of amphivasal bundles take place on the outside of the fibrovascular ring and not internally as in the buttercup. This situation seems to be rather a common one for the Umbelliferae as a whole. As the nodal region is passed the amphivasal bundles open up toward the outside of the stem and constitute dense, flat, fibrovascular segments of collateral organization. Similar conditions in regard to the resolution of the amphivasal strands are found in the buttercup.

Fig. 37 illustrates the organization of the stem in the nodal region of *Rumex* sp. The foliar traces are numerous, and to a large extent have passed into the cylinder. The periphery of the medullary region is occupied by a number of fibrovascular bundles of amphivasal organization. These are the result of the fusions of strands of the secondary and main axes by their phloems. The amphivasal strands in lower regions of the internode undergo fusions with the bundles of the cylinder, and finally completely disappear before the next node is reached. Fig. 38 shows a part of fig. 37 more highly magnified. The amphivasal strands can be seen on the medullary side of the collateral bundles which constitute the main cylinder.

These illustrations, which might be indefinitely multiplied from the nodes of herbaceous Dicotyledons, make it clear that amphivasal bundles resembling those of the Monocotyledons are frequently present in the nodal regions of dicotyledonous herbs, and result from the fusion of strands by their phloem faces. The similarity of the conditions in the two great divisions of the Angiosperms may be confirmed by an illustration taken from the grasses. Fig. 39 shows the nodal region of the upper part of the aerial stem of *Zea*. A leaf base is uniting with the stem, and on the upper left the fusion of the foliar vascular supply with that of the axis is farther advanced than it is on the lower right side of the illustration. This condition is due to the fact that either the section is not exactly transverse, or else the fusion of the leaf base is not effected in an exactly horizontal plane. It matters little which explanation be adopted. The bundles which enter the stem from the leaf are obviously extremely numerous, and their accommodation in the stem is accordingly a matter of some complexity. The device by which their reception is effected is by means of fusions similar to but more complicated than those exemplified in the dicotyledonous types figured. Fig. 40 shows a part of the upper left hand portion of fig. 3 more highly magnified. Three amphivasal bundles can be seen forming an oblique line from the lower left to the upper right of the figure. Between these lie other bundles which are in the act of fusing. A characteristic feature of the nodes of the aerial stem of grasses, sedges, and rushes is the presence of amphivasal bundles in the region of the nodes, resulting from the fusions effected in connection with the entry of the numerous foliar bundles into the stem. In the mass of Monocotyledons these amphivasal fusions are no longer found in the often highly specialized, annual, aerial axis, but are confined to the more primitive, perennial, subterranean stem. In the Scitamineae and true palms amphivasal bundles are usually entirely absent.

We may now turn to further illustrations of the absence of cambial activity in foliar strands which have recently entered the stem in the dicotyledonous Angiosperms. The legumes are a very important family of Dicotyledons, which are represented in temperate regions by many herbaceous forms. Fig. 41 shows the

infranodal region of the common garden bean. Since the lower part of the aerial stem is represented, the cylinder is somewhat thick and woody, as well as round in form. A leaf trace occupies the central region of the figure, and it is clearly marked by the absence of the dark cambial zone, which marks the stem bundles on either side. It is evident that absence of secondary activity is a feature of the leaf trace in a form which for many years has served in the laboratory for an example in studying the anatomy of the herbaceous dicotyledonous stem. As a further illustration the aerial axis of the common red clover (*Trifolium pratense*) may be used. Fig. 42 makes clear the conditions found in the nodal region of this species. A well marked black band indicates the cambial activity of the bundles of the stem proper. In the median region appears a leaf trace which is quite conspicuously without cambial activity, and in this respect presents a marked contrast with the cauline bundles which are adjacent to it on either flank. These illustrations, which might be indefinitely multiplied from the common leguminous types, clearly show that in this group the tendency toward the elimination of cambial activity in the leaf trace makes itself obvious.

The garden poppy will supply an example from another and somewhat distant group. In fig. 43 is shown the region of the node in the annual garden poppy of hybrid origin (*Papaver* cross). The foliar traces in many cases in this genus enter the stem as double strands. One of these pairs appears in the center of the figure, and it can easily be seen that its constituent strands are without the secondary activity due to the presence of the cambium, exemplified in the cauline bundles on either side of the foliar pair. Fig. 44 shows a total transverse section of the stem of the wild morning glory (*Convolvulus* sp.). On the upper side of the cylinder is shown a broad arc breaking away from the fibrovascular ring. This is the leaf trace of the leaf attached to the next node above. Nearly opposite, but somewhat obliquely placed, is a trace of a still higher leaf. It is clear that the foliar traces show a considerably less degree of secondary growth than is found in the fibrovascular tissues of the stem proper.

An examination of many nodes in herbaceous Dicotyledons has made it clear that there is a strong tendency for cambial activity to disappear in the leaf traces as they enter the cylinder of the stem. Even when the foliar trace does not intermit its secondary growth, there is always a considerable reduction of activity in this respect. This phenomenon, in fact, is not confined to Dicotyledons, but is also exemplified in Gymnosperms, both living and extinct, and in Cryptogams where secondary woody tissues are developed. There is an obvious advantage to the plant in the elimination or reduction of cambial activity in the foliar strands, since the food materials elaborated in the leaves are thus more certainly assured of transfer, without essential loss, to the storage regions of the stem. In the case of the herbaceous Dicotyledons, however, the much greater degree of assimilative activity causes so great an emphasis of the limitation of cambial activity in the leaf traces that in a large number of cases it disappears altogether. This feature, together with the usual multiplication of leaf traces in the more pronounced herbs, has produced in extreme instances among the Dicotyledons conditions which are not essentially different from those obtaining in the anatomical organization of the Monocotyledons.

In text fig. 1 is shown a stereodiagram of the stem of *Helianthus* in the region of a node. The node chosen is neither from the extremely upper region of the aerial stem, nor from its stout woody base. There are six leaf traces present, three facing, and three on the opposite side of the stem. Each trio of traces belongs to one of the opposite leaves. On the remote side the lateral traces are represented as passing into the leaf base, while the median is still in the axis. The nearer aspect of the stem is deprived of its superficial tissues, so that the relation of the traces to the organization of the wood may easily be seen. In the transverse aspect each leaf trace is clearly subtended and flanked by the storage tissues of the foliar rays. As the leaf rays descend in the stem they fork as a result of the intrusion of a median tongue of wood into the ray from below. Each foliar strand in the not too slender axis of *Helianthus* is accordingly related to a foliar ray which subtends it radially and at the same time accompanies it on either flank.

The accuracy of text fig. 1 may be confirmed by reference to figs. 7–14. It follows from simple geometrical considerations that as the stem becomes more slender in its upper portion, the radially subtending part of the foliar ray in the thicker region of the stem automatically disappears from view.

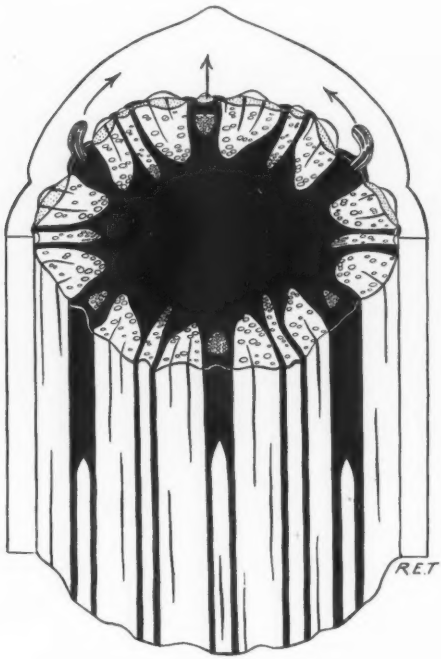

FIG. 1

The cambial conditions in dicotyledonous herbs are illustrated by text fig. 2, in which *a* represents the stouter lower region of the aerial axis of an herbaceous *Potentilla*. There are 5 outstanding segments of the stem, alternating with 5 depressed. The projecting portions of the wood cylinder show the presence of a cambium, indicated by a heavy line separating the xylem and phloem. In the depressed segments the wood has become largely parenchymatous storage tissue, subtended inwardly by the vascular foliar

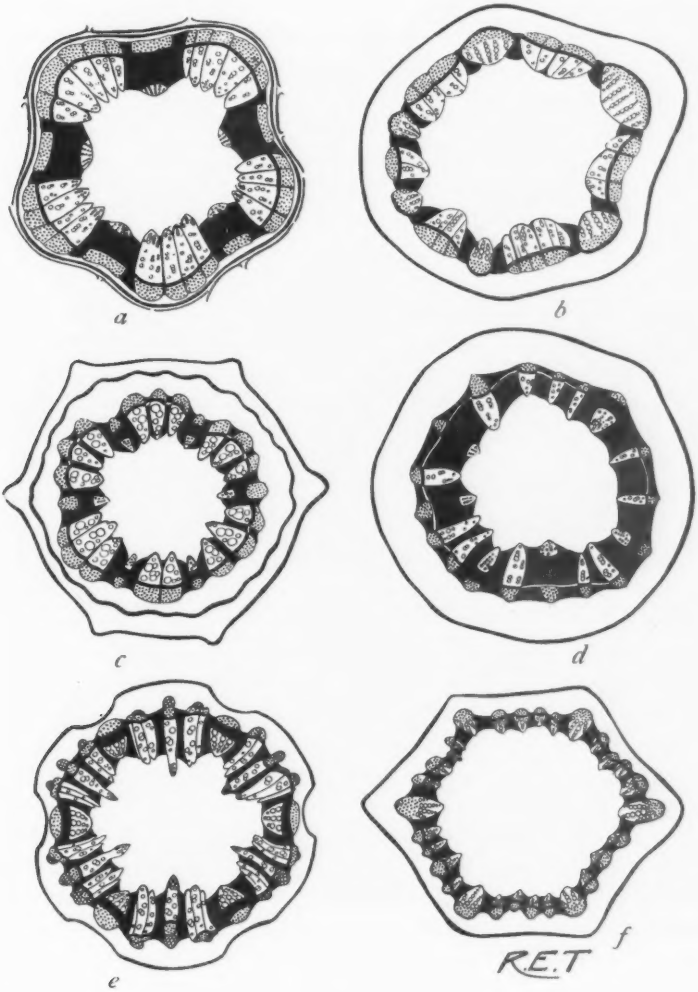

FIG. 2

traces. There is little or no cambial activity to be observed in these segments. In *b* is shown the upper slender region of the same aerial stem. Here the segments corresponding to leaf traces contrast with those in the former figure by their outstanding character. The absence of cambial activity, however, is to be observed as in the foliar segments of the stouter axis. The leaf traces are also more numerous than in the other instance, a condition not at all uncommon in the upper regions of herbaceous stems. This phenomenon, for example, is found in the common bean, and in many other cases. The depressed segments in the slender portion of the stem of *Potentilla* correspond to the outstanding ones in the thicker region nearer the ground. Cambial activity is indicated in the depressed portions of the stem separating xylem and phloem. The conditions in a woody axis of *Clematis* are shown in *c*. The depressed segments of the cylinder correspond in position to the foliar traces, and are without cambial activity. The outstanding arcs, on the other hand, represent the cauline bundle system, in which well marked secondary growth is present. In *d* is shown the subterranean perennial stem of *Actaea*, as an example of the essential similarity in organization of terrestrial axes to that found in the woody region of the aerial stems of herbaceous types. The broad, black, radial bands are the foliar rays. In proximity to a node the leaf ray is subtended by a broad radial band of storage tissue, and cambial activity, as represented by a line separating xylem and phloem, is absent. In the case of traces which are remote from their corresponding leaf in the vertical plane, cambial activity has been restored and the foliar storage parenchyma is divided into two by a woody central isthmus. This condition may well be compared with that shown in text fig. 1 for *Helianthus*. The topography of a moderately thick and woody node of the aerial stem of *Helianthus* is shown in *e*. The 6 foliar segments of the cylinder are depressed and show the absence of distinct cambial activity. The subtending portion of the foliar ray is only moderately well developed in this instance to correspond to the slight degree of thickening of the stem. In the outstanding portions of the cylinder a cambium is present and is conventionally represented by heavy lines separating xylem and phloem. In *f* is

shown an upper slender region of the stem in the sunflower. In accordance with the general principles formulated in the other examples, the foliar segments are here both outstanding and devoid of cambial activity. The remaining bundles are depressed and show the presence of secondary growth.

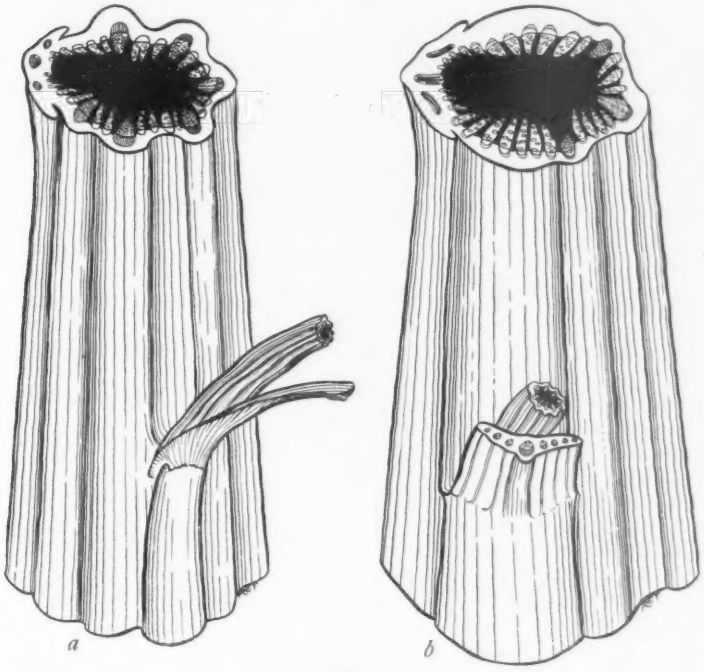

FIG. 3

Attention has already been given to the distribution of cambial activities in the nodal regions of herbaceous Dicotyledons. Another important factor in the development of the herbaceous type is shown in text fig. 3, which represents diagrammatically the stems of *Helianthus annuus* and *H. orgyalis*. In the former more herbaceous species a leaf base is represented on the left, at the upper end of the figure. This has 3 traces, as is normally the case in the genus. In addition to the 3 traces entering at the node, from the

leaf base are 6 others, emphasized in the diagram by the radial hatching of their outer regions. It is obvious that the traces of 3 leaves can be seen simultaneously in transverse sections of the stem. In other words, the leaf traces, together with their accompanying leaf rays, by their much elongated downward extension constitute an important topographical feature of the stem in many herbaceous Dicotyledons. In *b* is shown a corresponding diagram for *H. orgyalis*, a perennial species and one less typically herbaceous than *H. annuus*. Here only the traces of one other leaf can be seen. A feature of interest in the development of the herbaceous habit in *Helianthus* is the arrangement of the leaves. In the lower regions of intermediate types the leaves are opposite, while in the upper nodes the phyllotaxy is alternate. In the woody *H. hirsutus* the phyllotaxy is often opposite throughout. In *H. annuus* and *H. argyrophyllus*, annual and extremely herbaceous species, the leaf arrangement is generally entirely alternate.

Outstanding features of the organization of the extreme herbaceous type illustrated by the Monocotyledons are the occurrence of medullary strands and amphivasal fibrovascular bundles. These features are likewise present in extreme herbs in the dicotyledonous series. Text fig. 4 illustrates these characteristics for the two divisions of the Angiosperms. In *a* is shown the stem of *Sanicula* in the region of the node, where the leaf base is just uniting with the axis. An axillary bud is represented by its fibrovascular cylinder. The inner bundles of this are uniting by their faces with the opposite bundles of the main axis in such a manner as to give rise to typical amphivasal strands. These are clearly shown in *b*, which represents a lower plane of section than *a*. On the upper side of the cylinder are a number of amphivasal strands resulting from fusions between the bundles of axis and branch. In *c* the amphivasal strands have become collateral once more and the numerous foliar traces are seen entering the stem. In *d* is shown the nodal region of *Rumex* sp. The same method of formation of amphivasal strands is seen as in *Sanicula*, namely, by fusions between opposed strands of axis and branch. In this case the concentric bundles are so numerous as to be brought into the medullary region. In *e* is represented a lower plane of section

with a greater number of amphivasal bundles. In *f* the strands in the pith have reached their maximum number and are beginning to undergo fusions with the bundles of the wall of the cylinder.

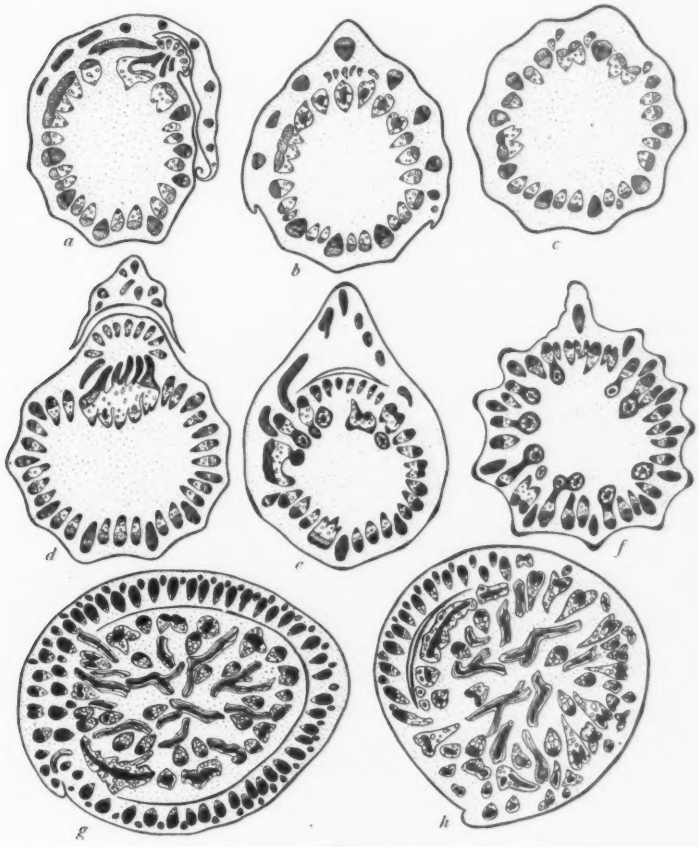

FIG. 4

The foliar traces are very numerous here as in *Sanicula*. In *g* and *h* are represented nodal sections of *Zea*. In *h* the leaf base has just fused with the axis and shows a very large number of bundles. In the central region of the stem many horizontal and vertical

leaf base are 6 others, emphasized in the diagram by the radial hatching of their outer regions. It is obvious that the traces of 3 leaves can be seen simultaneously in transverse sections of the stem. In other words, the leaf traces, together with their accompanying leaf rays, by their much elongated downward extension constitute an important topographical feature of the stem in many herbaceous Dicotyledons. In *b* is shown a corresponding diagram for *H. orgyalis,* a perennial species and one less typically herbaceous than *H. annuus.* Here only the traces of one other leaf can be seen. A feature of interest in the development of the herbaceous habit in *Helianthus* is the arrangement of the leaves. In the lower regions of intermediate types the leaves are opposite, while in the upper nodes the phyllotaxy is alternate. In the woody *H. hirsutus* the phyllotaxy is often opposite throughout. In *H. annuus* and *H. argyrophyllus,* annual and extremely herbaceous species, the leaf arrangement is generally entirely alternate.

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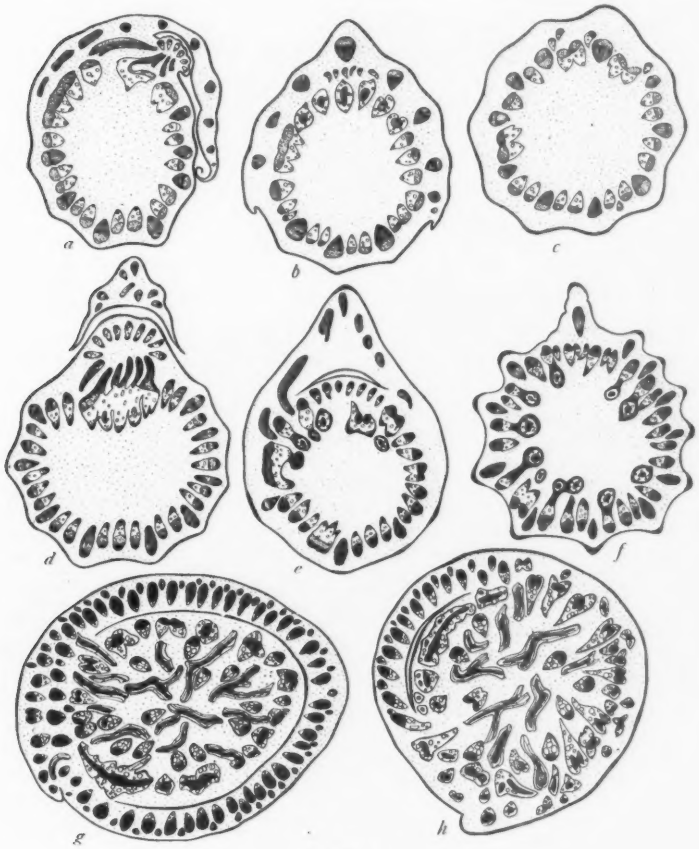

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unions of bundles are taking place, with the initiation of some amphivasal strands. In *h* the fibrovascular union of leaf and axis has advanced still farther, and many amphivasal bundles are present in the stem as a consequence.

It seems clear from these examples that the entry of numerous foliar strands at the node is correlated with the formation of amphivasal medullary strands. In most Dicotyledons these strands are the result of the facial fusion of the bundles of axis and branch, but such fusions are usually associated with a large number of foliar traces simultaneously entering the stem at the node. In the Monocotyledons the amphivasal strands result from the fusion of stem bundles with one another, or from the fusion of foliar traces with those of the stem in the region of the node. The amphivasal strand so characteristic of many higher Dicotyledons and the lower Monocotyledons probably originated in the first instance as a consequence of the fusions of stem bundles with stem bundles, and later, with the still further multiplied number of foliar traces characteristic of the Monocotyledons, by fusions of leaf bundles with stem bundles.

Conclusions

The various data introduced in this paper appear to justify the drawing of certain general conclusions. First of all, statements made as to the absence of foliar rays in herbs are not consistent with the facts of anatomy. Such rays are so characteristic a feature of organization of the more woody region of the aerial axis of herbs that they constitute a well marked diagnostic feature of such forms. The foliar rays of herbs are the result of the aggregation and fusion of the ordinary rays of woody stems, in relation to the foliar traces. Accompanying and characteristic of the process of fusion is the transformation of the vessels into fibers and the septation of the latter in turn into parenchymatous elements. The final consequence of this activity is the formation of large masses of storage tissue in relation to the incoming foliar traces. By comparison of nearly related species of the same genus, which are progressively more herbaceous, the following interesting conditions can often be observed. The accentuation of the herbaceous habit is accompanied by marked increase in the size of the leaf trace and of the

foliar ray to which it is related. Further, the foliar ray becomes much more homogeneously parenchymatous in more advanced and herbaceous species, and does not contain the admixture of vascular and fibrous elements which reveal the mode of origin of the foliar rays in more woody and primitive species.

The foliar ray surrounding and subtending the leaf trace is characteristic of the less advanced dicotyledonous herbs and of the lower more woody region of those higher in the herbaceous sequence. This type of ray gives place, by the later thinning of the woody cylinder, to one in which the storage tissue is confined to the flanks of the traces. The long vertical extension of these flanking rays results in the division of the originally continuous woody cylinder of the ancestral Dicotyledons into a circle of separate strands, the fibrovascular bundles. Of the strands thus resulting, those more closely related to leaves manifest an interesting contrast to the others because they very frequently manifest an absence of cambial activity. This cambial inactivity seems to be a safeguard against the undue consumption of assimilates in the growth in thickness of the foliar strands. Such growth in thickness would clearly not be advantageous to organisms dependent for their success in the struggle for existence on the amount of food stored up either in their stems or their seeds. The correctness of this interpretation of the undoubted fact that foliar traces lose or tend to lose their cambial activity in the stem of herbaceous Dicotyledons is vouched for by conditions observed in roots, to be enlarged upon in another connection. Roots permanently and perennially provided with root hairs are usually without secondary activity in the woody cylinder, while roots of allied species without persistent root hairs have the secondary tissues well developed. In other words, the more efficiently absorptive roots are without secondary growth, while roots of allied woody and less efficient species are well provided in this respect. In advanced herbs, such as the members of *Ranunculus*, the traces of leaves and roots stand out conspicuously in the subterranean stem by the absence of the secondary growth characteristically present in the fibrovascular strands of the stem proper.

It appears clear from these considerations that the herbaceous type is the extreme expression of efficiency, and that the correlated

reduction in or complete loss of secondary growth is physiologically advantageous to the plant.

An incidental and probably less essential modification in extreme herbs is the concurrence of medullary and amphivasal strands. These are apparently the result of the entry of very numerous bundles at the nodes, a feature of many advanced and angiospermous herbs. The resulting difficulties of accommodation are most readily overcome by the scattering and fusion of the crowded strands. In lower types the amphivasal and medullary bundles are the consequence of the fusion of facing cauline bundles of axis and lateral branch. In the highest herbs the foliar traces are concerned in the formation of medullary strands and in the scattered distribution of bundles throughout the transverse sections of the stem.

Summary

1. Herbaceous Dicotyledons have developed from arboreal dicotyledonous types by the formation of storage rays about the leaf traces.

2. In the more primitive herbs the foliar rays are shallow longitudinally, but of considerable radial depth.

3. In higher herbaceous Dicotyledons the foliar rays lose in radial dimensions as a result of the thinning of the woody cylinder, but this loss is largely compensated for by their increasing vertical extension, which often carries them through several internodes.

4. In the higher herbaceous Dicotyledons the foliar traces tend to multiply in number with the increased efficiency of the leaf.

5. Another important development in advanced dicotyledonous herbs is the progressive disappearance of cambial activity in the foliar trace, which often, in spite of this, is larger in size than the bundles of the stem.

6. The increase in number and importance of the foliar traces, as well as the greater relative importance of secondary axes in high herbs, leads to the crowding of strands at the node, which in turn results on the one hand in scattered distribution of the bundles in the stem, and on the other to formation of amphivasal strands.

7. The disappearance of secondary growth in foliar traces of advanced dicotyledonous herbs appears to be explainable on the grounds of physiological advantage.

8. Absence of secondary growth extends from the leaf traces to the rest of the bundles situated in the stem, and a condition practically monocotyledonous results.

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EXPLANATION OF PLATES I–VII

PLATE I

FIG. 1.—Transverse section of lower region of aerial stem of *Aster novae-angliae*, slightly magnified to show presence of foliar rays on upper left hand.

FIG. 2.—Transverse section of extreme upper slender portion of stem of *Aster novae-angliae*, slightly magnified to show alternation of leaf traces and leaf rays with ordinary stem bundles (approximately 8 of each).

FIG. 3.—Part of fig. 2, more highly magnified.

FIG. 4.—Part of fig. 2, more highly magnified.

FIG. 5.—Part of fig. 2, more highly magnified.

FIG. 6.—Leaf trace and subtending and flanking storage tissue, from slender upper portion of stem of *Aster novae-angliae*.

FIG. 7.—Leaf ray of *Helianthus annuus* in transverse section, slightly magnified.

PLATE II

FIG. 8.—Transverse section of foliar ray of *Helianthus orgyalis*, moderately magnified.

FIG. 9.—Longitudinal section of foliar ray of *H. tuberosus*, somewhat highly magnified to show mixed organization consisting of rays, fibers, and vessels.

FIG. 10.—Tangential section of leaf ray of *H. hirsutus*, showing it as yet incompletely aggregated from elements of ordinary wood and consequently consisting of an obviously mixed assemblage of wood rays, vessels, and fibers.

FIG. 11.—Tangential section of foliar ray of *H. tuberosus*, showing more advanced condition of foliar ray in more advanced herb; ray is broader, leaf trace larger, and structure more homogeneous than in fig. 10, which has same degree of magnification.

FIG. 12.—Foliar ray of *H. annuus*, very marked herbaceous species of the genus; magnification same as in two preceding figures, and shows that leaf trace is much larger, as is also the accompanying ray, which is further marked by a much greater advance toward homogeneity than two foregoing figures.

FIG. 13.—Part of fig. 12, more highly magnified to show presence of some degree of variety in elements composing foliar ray; magnification same as fig. 9, of *Helianthus tuberosus*.

FIG. 14.—Magnified view of part of fig. 10, representing ray of *H. hirsutus;* for purposes of comparison magnification is identical with figs. 9 and 13.

PLATE III

FIG. 15.—Transverse section of one of angles of upper region of aerial stem of *Helianthus tuberosus*, showing leaf trace in center flanked by stem bundles on either hand.

FIG. 16.—Right hand stem bundle of last figure, more highly magnified to show presence of cambial activity between xylem and phloem.

FIG. 17.—Transverse section of leaf trace in fig. 15, more highly magnified to show absence of cambial activity in foliar strand.

FIG. 18.—Transverse section of mature root of *Aster Shortii*, showing pentarchous organization with well developed secondary growth; root hairs have disappeared.

FIG. 19.—Transverse section of younger root of *A. Shortii*, about phase when secondary growth is beginning; root hairs still present but beginning to wither away.

FIG. 20.—Transverse section of persistently hairy root of *Aster umbellatus;* continued presence of root hairs correlated with absence of secondary growth.

PLATE IV

FIG. 21.—Transverse section of very herbaceous species of *Aster, A. tataricus;* normal woody structure of *Aster* replaced by herbaceous texture and by more foliar traces (7) than those usually present in species of the genus (3).

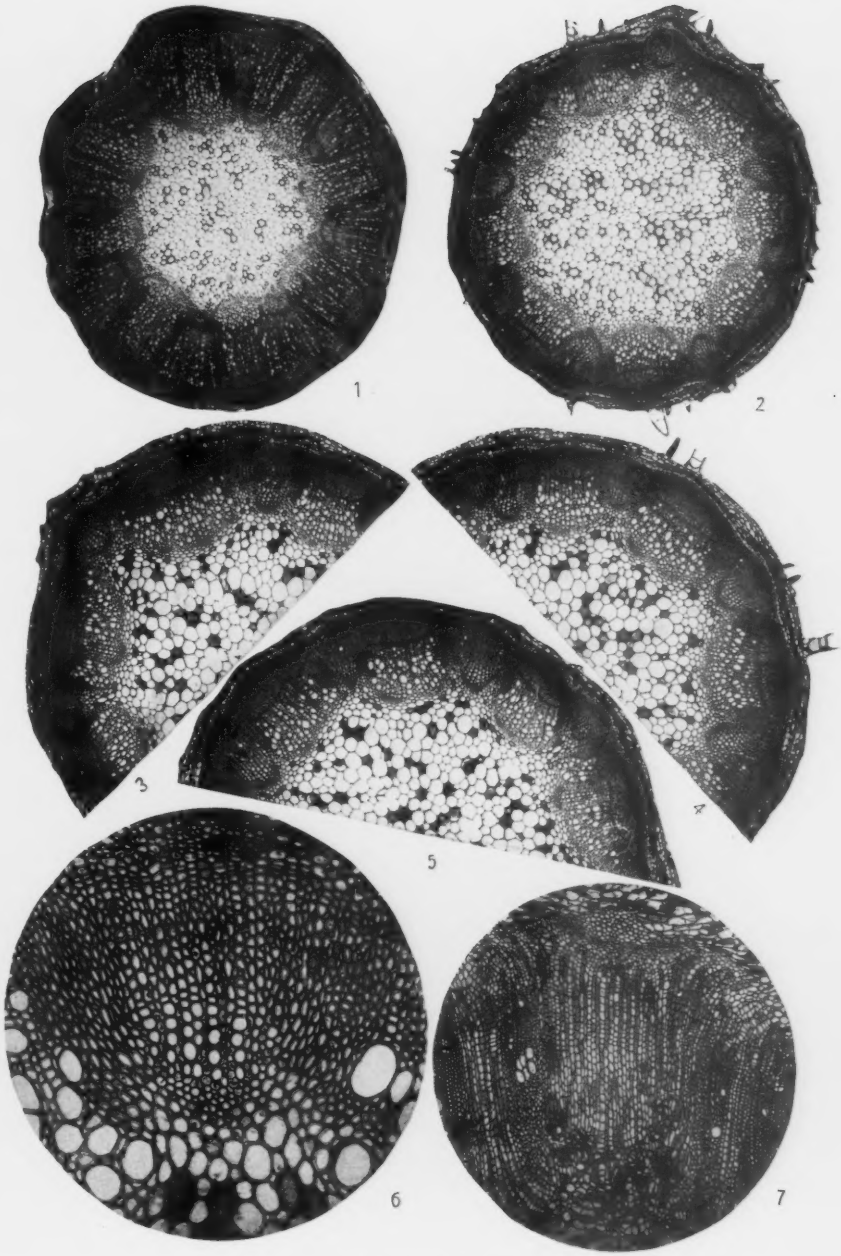
FIG. 22.—Transverse section of small branching stem of *Ranunculus acris*.

FIG. 23.—Transverse section of nodal region of larger branching stem of *R. acris*.

FIG. 24.—Transverse section of stem of *R. acris*, below node; 5 foliar traces in axis appear darker than stem bundles by reason of their vessels being occupied by gummosis.

FIG. 25.—Part of section similar to that shown in fig. 24, more highly magnified to show characteristic difference between foliar and cauline bundles.

FIG. 26.—Another of the same, showing different bundles.



JEFFREY and TORREY on ANGIOSPERMS

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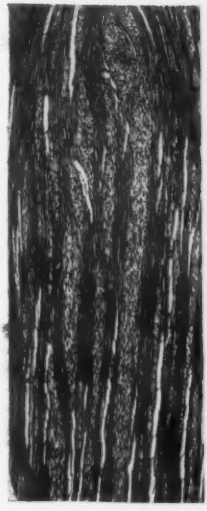

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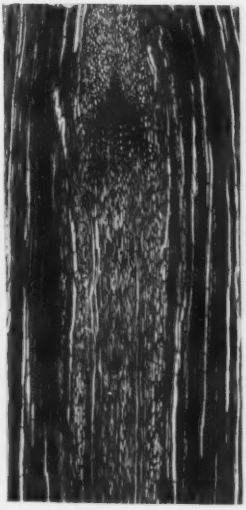

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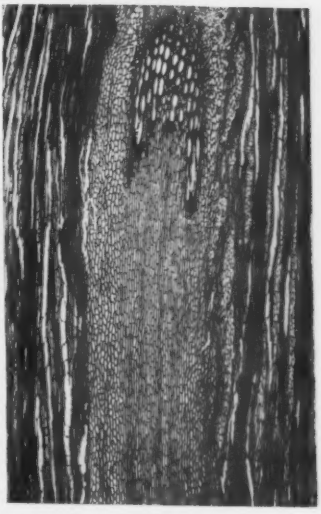

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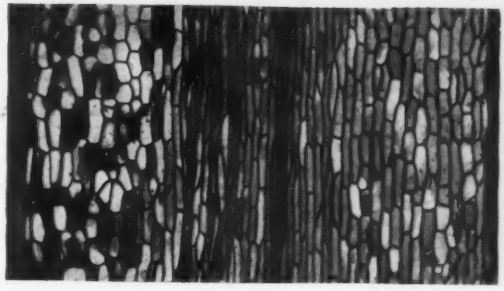

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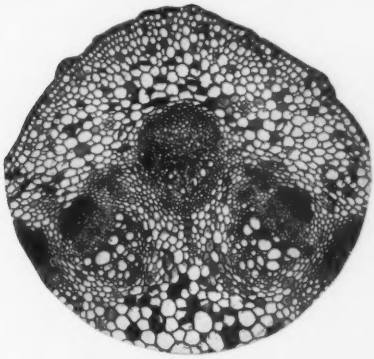

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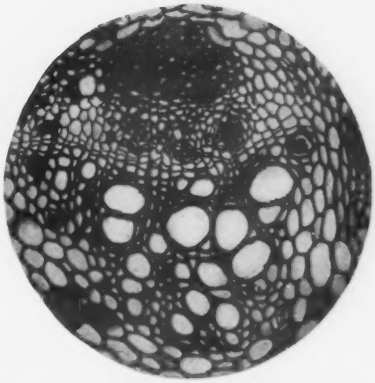

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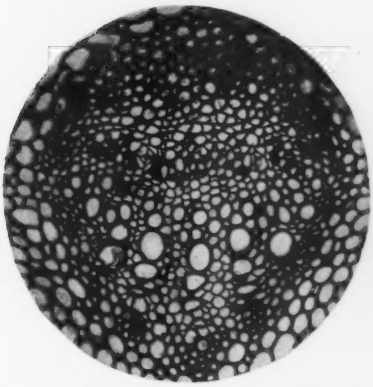

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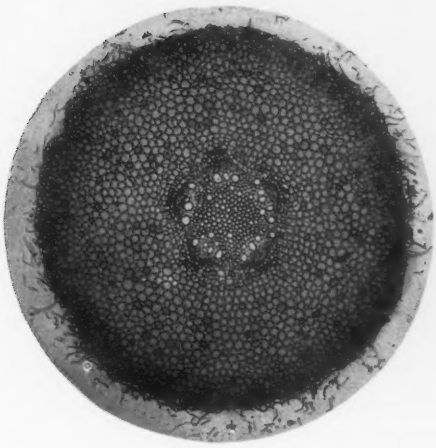

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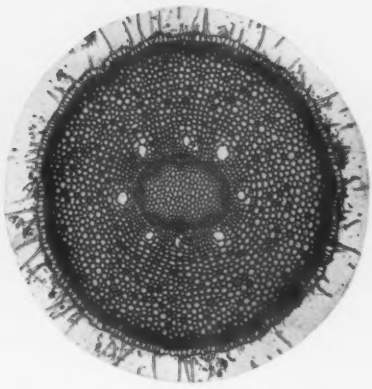

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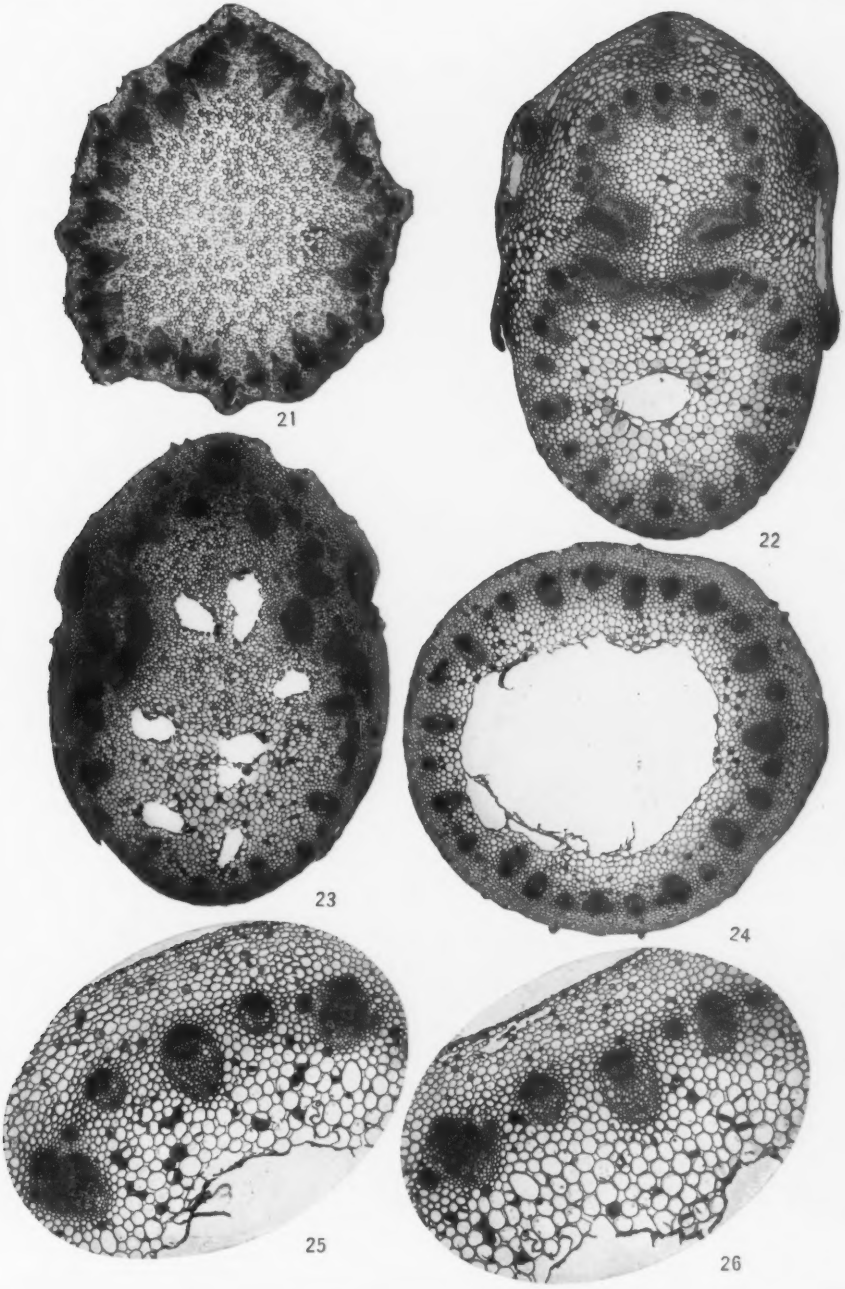

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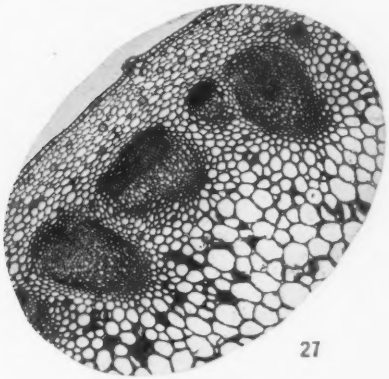

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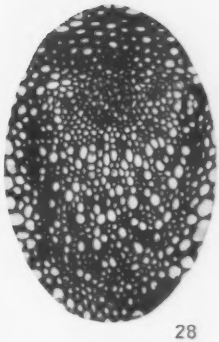

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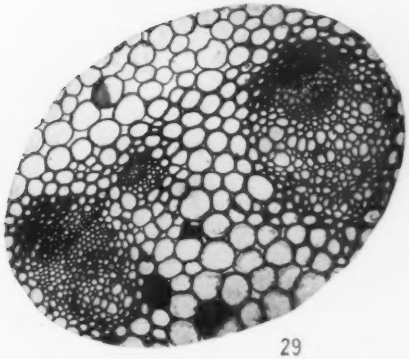

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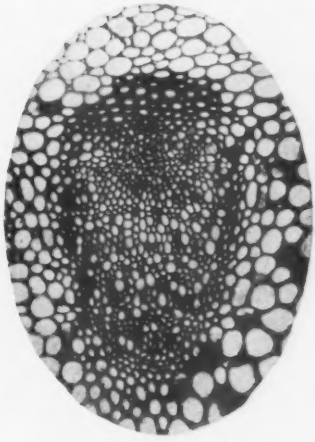

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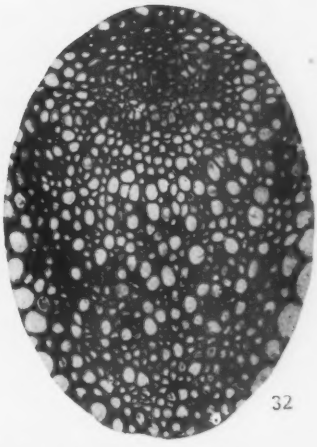

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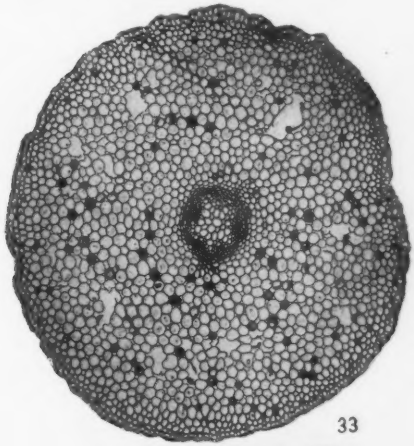

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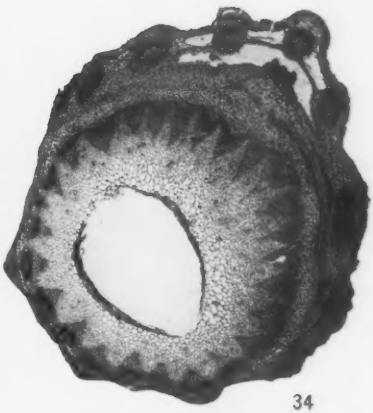

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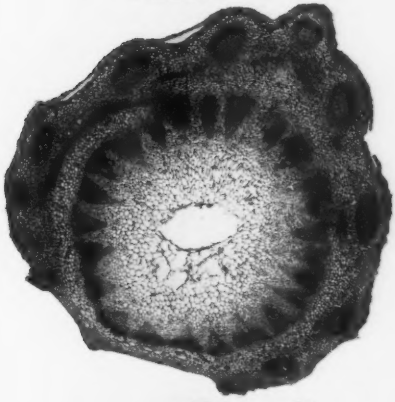

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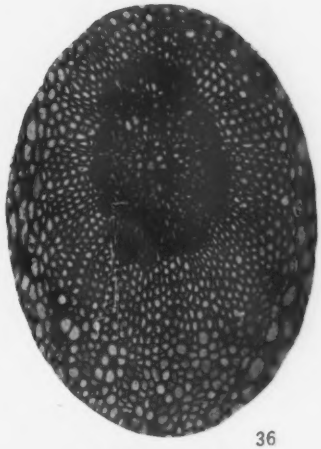

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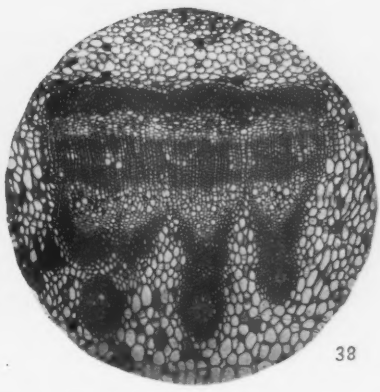

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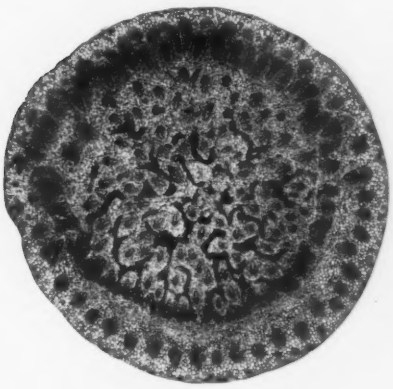

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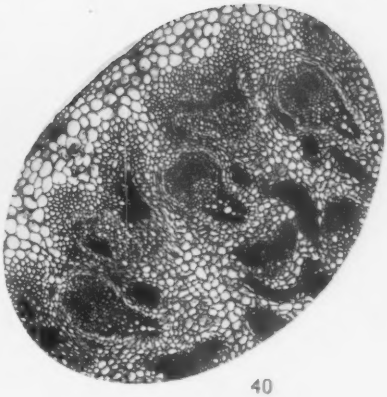

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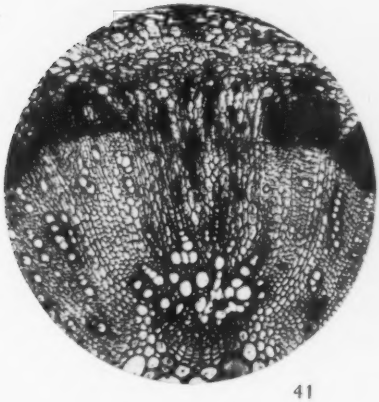

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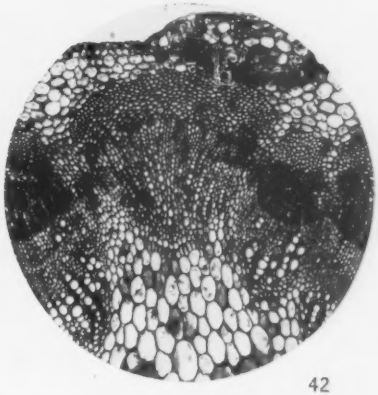

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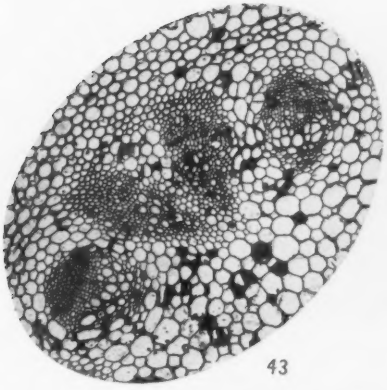

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JEFFREY and TORREY on ANGIOSPERMS

PLATE V

FIG. 27.—Part of transverse section of infranodal region of stem of *R. acris*, showing bundles under slightly higher magnification than in previous two figures.

FIG. 28.—Foliar trace of *R. acris*, somewhat more highly magnified to show absence of cambial activity.

FIG. 29.—Leaf (left) and stem bundle (right) of *R. acris*, showing absence and presence of cambium.

Fig. 30.—Leaf trace of *R. acris*, somewhat highly magnified to show absence of cambial activity.

FIG. 31.—Stem and leaf bundles of *R. acris*, moderately magnified.

FIG. 32.—Leaf trace of *R. acris*, somewhat highly magnified to show absence of cambium.

PLATE VI

FIG. 33.—Transverse section of old root of *R. acris*, showing absence of cambial growth.

FIG. 34.—Transverse section of stem of *Sanicula*, in region of branching node.

FIG. 35.—Transverse section of same, slightly lower down in axis than in previous figure.

FIG. 36.—One of amphivasal concentric strands occurring in nodal region of stem of *Sanicula*.

FIG. 37.—Transverse section in region of node of *Rumex*, showing presence of numerous medullary amphivasal strands, resulting from fusion of bundles in region of node.

FIG. 38.—Part of fig. 37, more highly magnified to show details of organization.

PLATE VII

FIG. 39.—Transverse section through upper node of *Zea*, showing fusion of leaf base and bundles with corresponding structures in axis.

FIG. 40.—Part of upper left hand portion of fig. 39, more highly magnified to show presence of amphivasal strands resulting from fusions in region of node.

FIG. 41.—Foliar segment from lower woody region of axis of wax bean, showing absence of cambial activity in region of foliar trace.

FIG. 42.—Transverse section through foliar segment of red clover, showing absence of cambial activity in foliar trace.

FIG. 43.—Transverse section through nodal region of *Papaver* sp., showing two leaf traces in center, in contrast to stem bundles, on either side, by absence of cambial activity.

FIG. 44.—Transverse section of stem of *Convolvulus* sp., showing reduction of cambial activity in leaf segments as contrasted with those of stem.

AFTER-RIPENING AND GERMINATION OF JUNIPERUS SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 275

DEAN A. PACK

(WITH ONE FIGURE)

Some seeds fail to germinate in compensating percentages or even at all when placed under ordinary germination conditions. Because of inquiries directed to this laboratory from various growers concerning the best methods of handling juniper seeds, there was conducted a careful study of after-ripening, germination, and seedling development, as well as some of the chemical and physiological changes involved in these processes. Strict quarantine laws, recently put into effect, will mean that many species of decorative plants that were formerly grown from seeds in foreign countries and brought to America as plants, must now be grown from seeds by American nurserymen. This will doubtless promote study of the germinative behavior of many refractory seeds in the future.

Literature

Wild plants of the temperate zone produce seeds that usually have a rest period, which varies as to length and cause with the different species and kinds of seeds. This dormancy is found to be characteristic of the seeds of 75 per cent of the wild and the cultivated plants studied by HOWARD (**18**). Although the rest period of most seeds is only a few months, it may be years, as in the case of some Conifers (**21**) and of *Euphorbia Cyparissias* (**19**). CROCKER (**5**) states that delayed germination is due to one or more of the following conditions: (1) rudimentary embryo, (2) dormant embryo, (3) coats inhibiting embryo expansion, (4) coats inhibiting gas exchange, (5) coats inhibiting water absorption, (6) a combination of two or more of these, and (7) secondary dormancy. Up to date seeds have been studied that represent each of these different types of dormancy.

As it has been impossible to dispense with this rest period in all cases, many substances have been used to reduce dormancy and force seeds to germinate. Concentrated sulphuric acid has been used by HILTNER and KINZEL (17), ROSTRUP (32), and others with positive results. Among the salts ROSE (31) noted that the sulphates and nitrates were the better forcing agents. Hydrogen peroxide and increased oxygen pressure forced the germination of *Xanthium* seeds (5). Wounding and treatment with ether stimulated the germination process (3). Light has been found to force or to inhibit germination depending on the seed (12, 20). The New York Experiment Station (24) and many others have shown that desiccation improves the germinating power of corn. The hot bath has been used with success on some seeds (4). Alternating temperatures have been used to force grass seeds in the Seed-testing Laboratories of the Bureau of Plant Industry. With these much has been claimed for freezing and thawing as a forcing agent (29). LAKON (21), however, found that the germination of *Pinus Peuce*, *P. Cembra*, *P. Strobus*, and *P. silvestris* could not be accelerated by treatment with dry heat, warm bath, file injury, ether, chloroform, salt solutions, concentrated sulphuric, or dilute acids.

Seeds with dormant embryos must go through a series of changes (after-ripening) before germination can occur (5). The after-ripening of hawthorn seeds proceeds fastest at 5–6° C. according to DAVIS and ROSE (8). An idea of this after-ripening process may be gained by following the results of LAKON on a protein and ECKERSON (10) on a fatty seed. LAKON (22), in studying the changes that precede germination of *Fraxinus excelsior*, found very little increase in water absorption. From the tenth day on, starch accumulated in the embryo cells, with a corresponding disappearance of protein from the endosperm cells. In place of the disappearing protein a turbid emulsion formed, which later was digested. At no time did starch appear in these endosperm cells. The embryo doubled its length during this process of "Vorkeimung." ECKERSON (10) studied the changes occurring in the hawthorn seed during after-ripening, and reported an increasing acidity and water absorbing power of the dormant organ. The catalase, peroxidase, and oxidase activity increased as after-ripening and

germination proceeded. Germination was accompanied by a decrease of stored fats and an increase of sugar. Although the details varied somewhat, both seeds passed through a period of preparation for germination.

Material and preliminary study

The *Juniperus* plants are erect or prostrate dioecious Cupresseae distributed over the Northern Hemisphere. They are used in landscape decoration, serving as hedges and screens up to 30 ft. high. In early spring the flowers appear in the leaf axils, forming many carpel whorls, of which only the upper one develops. This whorl bears 3 ovules, which grow together and form a spherical fruit, which requires two years to ripen, and contains 1–3 seeds.

TABLE I

MATERIAL SECURED

Species	Lot	Date	Place
J. virginiana L.	1	November 11, 1918	West Newberry, Massachusetts
J. c. depressa Pursh.	2	January 1, 1919	Boxford, Massachusetts
J. communis L.	3	January 1, 1919	Vermont
J. prostrata Pres.	4	January 1, 1919	Vermont
J. virginiana L.	5	January 1, 1919	Vermont
J. communis L.	6	April 19, 1919	Near Chicago, Illinois
J. virginiana L.	7	April 19, 1919	Near Chicago, Illinois
J. communis L.	8	September 19, 1919	Near Chicago, Illinois
J. virginana L.	9	September 19, 1919	Near Chicago, Illinois

Juniperus seeds were gathered in the fruit condition, and those used in these experiments were collected as stated in table I.

The seeds freed from the fruit vary with the species as to color, shape, size, and quality. Those of *J. virginiana* are light brown, smooth, brittle, 3–4 mm. long, and when air-dry weigh about 0.009 gm. each. Seeds of *J. c. depressa*, *J. communis*, and *J. prostrata* are much alike. These seeds are dark amber, rough, 4–6 mm. long, narrower and less brittle than those of *J. virginiana*. Some of the *J. virginiana* material proved to be badly worm eaten, while the other lots were quite free from worms. Seeds collected in Vermont were generally good. Table II gives the percentage of bad seeds due to worms and lack of development.

Fig. 1 shows the structure of the seed of *J. virginiana*, with its many membranes and protective layers. In strong contrast with the hard brown coat are the clear white endosperm and embryo. The hard coat consists of three layers: the outer fleshy (*a*), the stony (*b*), and the heavy inner fleshy (*c*). In the outer fleshy layer are found pectic substances and methyl pentosans. The stony layer is lignified and contains other substances, as calcium, pectates, and pentoses. The inner fleshy layer is well developed and consists of suberin with some little cellulose. Of the endosperm, embryo, etc., one distinguishes the nucellus (*d*), the mass of distorted tissue (*e*), the hypocotyl cap (*f*), the megaspore membrane (*j*), the endosperm wall (*k*), the endosperm (*g*), and the embryo (*h*).

TABLE II

PERCENTAGE OF IMPERFECT SEEDS IN LOTS 1, 3, 4, AND 5

Species	Lot	No. examined	Percentage imperfect
J. virginiana	1	100	59
J. virginiana	1	50	63
J. virginiana	1	100	61
J. virginiana	1	2000	60
J. communis	3	1000	26
J. prostrata	4	1000	20
J. virginiana	5	2000	22.5

The nucellus is constructed of long narrow cells which give tests for cellulose and pectic acid. The mass of tissue (*e*) protecting the hypocotyl consists of cellulose, pectic substances, and some other groups of substances such as fats and gums. Between this mass and the hypocotyl is a cap of very fine and firm cells (*i*), which are made up of cellulose and hemicellulose. The megaspore membrane (*j*) is very thin and stains with ruthenium red. Examination shows that the outer wall of the outer layer of endosperm cells has been developed into a suberin wall (*k*). This wall is insoluble in concentrated H_2SO_4, 50 per cent chromic acid, and gives the phellic acid reaction. The endosperm cell walls are rather thick and made up of cellulose. Cell walls of the embryo are thin and consist of pectose and cellulose.

The storage substances of the resting seed are mentioned here, but they are later taken up in detail with the changes accompanying germination. Tannin is generally distributed throughout the coat. Some is stored in the nucellus, endosperm wall, and hypocotyl cap, but none was found in the endosperm or embryo. The embryo and endosperm are stored with an abundance of protein and fat, and also a trace of glucose. No starch was found in either endosperm or embryo. Histidine, tyrosine, and arginine are found in both endosperm and embryo. There is also a trace of leucine and probably cystine.

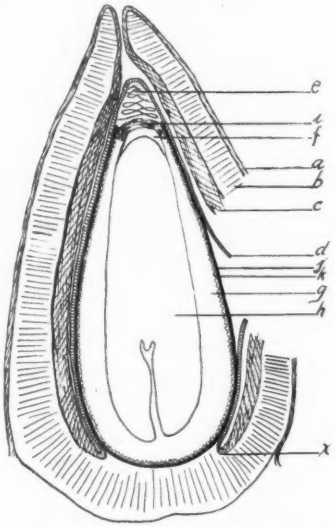

FIG. 1.—Longitudinal section of seed of *Juniperus virginiana* with part of nucellus and integument removed from one side: *a*, outer fleshy; *b*, stony; *c*, inner fleshy; *d*, nucellus; *e*, distorted tissue; *f*, hypocotyl cap; *i*, protective cap; *j*, megaspore membrane; *k*, endosperm wall; *g*, endosperm; *h*, embryo.

Catalase activity of the embryo and endosperm is low, while that of the coat is negligible. The seed shows peroxidase activity, with a mere trace of oxidase activity.

The resting seed embryo has a P_H value of about 8, while the endosperm has a P_H value of about 5. Thus the embryo is basic, while the endosperm is acid, a condition opposite to that usually found in seeds which are ready for germination.

Treatment of material

After collection the larger part of the fruit or berry was removed from the seed by running the berries through a coffee mill so wide open as not to injure the seed. Next the seeds were sifted and the milling and sifting repeated. The seed material was then rubbed between two sieves in the presence of an abundance of water. In this way all the berry and excess tissues which prevent

sterilization were easily and quickly removed. The bad seeds were floated off with water, and the good seeds rinsed and permitted to dry before sterilization.

After some testing, a 5 per cent solution of formalin acting for 2.5 minutes was selected as the best sterilizing agent for juniper seeds. It was found that formalin did not readily penetrate the coat, reduce the catalase activity, or hinder germination. The permeability of the coat was studied as follows. Seeds were submerged in different solutions for a definite time, removed, washed in distilled water, the coats removed, the seeds sectioned with a freezing microtome, and the sections tested for the respective solutions. Table III shows that the coat was very permeable to water, bases, and salts, but not permeable to stains and acids.

TABLE III

PERMEABILITY OF COATS TO WATER, STAINS, ACIDS, BASES, FORMALIN, AND SALTS

Substance	Permeability	Substance	Permeability
Eosin (dilute).....	Impermeable	Water.....	Very permeable
Eosin (strong).....	Impermeable	C_2H_5OH.....	Very permeable
Neutral red (dilute)..	Impermeable	NaOH.....	Very permeable
Neutral red (strong).	Impermeable	KOH.....	Permeable
Formalin.....	Slowly permeable	NH_4OH.....	Permeable
HCl N/100.....	Very impermeable	$AgNO_3$.....	Very permeable
H_2SO_4 N/100	Very impermeable	$HgCl_2$.....	Very permeable

That the salts ($AgNO_3$ and $HgCl_2$) penetrated the coats is shown by the catalase activity of the seeds with coats removed (table IV). These seeds, after being sterilized, washed, and incubated at 9° C. for 48 hours, had coats removed, and were ground for catalase activity determinations. It was further shown that $AgNO_3$ penetrated the coats by the fact that seeds so treated were killed. Seeds sterilized in formalin germinated, and therefore most of the seeds used in these experiments were sterilized 2.5 minutes in 5 per cent formalin. In this connection it should be noted that SCHROEDER (33) and GROVES (14) found that the coat of the wheat seed was practically impermeable to $AgNO_3$, and that this solution was a good sterilizing agent for wheat. This shows how the permeability of seed coats may vary with different seeds.

For some experiments the seeds were freed from the probable inhibiting influence of the hard coats by one of the three following treatments: (1) dry seeds were put into concentrated H_2SO_4 and the rate of penetration followed by testing with congo red; it required 24 hours to entirely carbonize the coat; the carbonized coats were rubbed off with filter paper and the seeds rinsed in a suspension of $CaCO_3$ and distilled water; (2) seed coats were also removed with seed nippers; (3) in other experiments only the end of the coat was cut away. The sterile seeds were put into sterile wide mouthed bottles, Petri dishes, or flasks for germination. Those cultures

TABLE IV

EFFECT OF STERILIZING AGENTS ON CATALASE ACTIVITY OF SEEDS, TREATED 2 MINUTES (40 SEED COATS REMOVED)

STERILIZING AGENTS	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
Water (check)	9.0	20.0	24.3
Water (check)	8.9	19.0	24.0
Formalin 5 per cent	8.5	21.0	24.8
Formalin 5 per cent	8.8	21.2	24.4
$AgNO_3$ 2 per cent	3.0	6.0	8.0
$AgNO_3$ 2 per cent	3.1	6.4	8.1
$HgCl_2$ 1 per cent	4.0	11.2	13.0
$HgCl_2$ 1 per cent	4.1	10.0	11.8

which required good ventilation were protected against infection by a system of tubes plugged with cotton. The seeds were left on the moist walls of the containers or on moist filter paper, depending upon the conditions of the experiments. In the determination of the effect of solutions as forcing agents, no foreign absorbing material was allowed in the flasks with the seeds. In all other cases, except where mentioned, the seeds were placed on moist filter paper.

Forcing agents

The change of the catalase activity and the ability of the seeds to germinate were used as standards to determine whether or not the substance or treatment under examination was a forcing agent for the juniper seed.

METHODS.—As it has been shown that catalase activity increases with after-ripening of the dormant-embryo seeds (6, 10), catalase activity was chosen as the first standard. Germination, the production of independent seedlings, was selected as the final standard. Great care was found necessary in the preparation of material and the manipulation of the catalase apparatus. As the berry has a high catalase activity, every trace of fruit was removed before grinding. The grinding was carried out under similar conditions, and with a definite amount of water and no. 2 sand per unit weight of seed material. The dioxogen was neutralized with N/10 NaOH at the time of using. All determinations were made with the bath at 25° C., and the drive wheel of the apparatus regulated to make 30 revolutions per 10 seconds. No explanation is needed for germination as a standard.

FORCING AGENTS.—Among the common forcing agents tried on the juniper seeds were high temperatures, alternating temperatures, removal of coats, hydrogen peroxide, dilute acids, carbon dioxide, light, soil, mercuric chloride, ether, and oxygen. The first seven of these had very little effect on the catalase activity and did not force germination. While the treatments with mercuric chloride, ether, and oxygen did not force germination, each had its influence upon catalase activity.

CROCKER and HARRINGTON, in an unpublished work at the Seed-testing Laboratories of the Bureau of Plant Industry, have found that $HgCl_2$ was a good forcing agent for Johnson grass. Juniper seeds were sterilized and put into flasks containing the following concentrations of $HgCl_2$. After 24 hours the excess of liquid was poured off. The results are given in table V. These data, as well as those obtained by grinding the seeds for catalase activity in the same concentrations, show that $HgCl_2$ reduced catalase activity in the higher concentrations. None of the seeds treated with $HgCl_2$ germinated.

In studying the effect of ether, seeds were sterilized, put into Petri dishes without covers, and exposed to air containing various amounts of ether by sealing in 9 liter cans. After a certain exposure the seeds were removed, aired, and placed to germinate. Table VI gives the catalase activity for seeds that were exposed to ether

6 days and then left in the germinator 95 days at 25° C. Seeds similarly treated, except that they were exposed only 2 days, gave less marked catalase activity. The point to be noted in table VI is the increased activity which was given by seeds treated with the larger amounts of ether. Of added interest is the fact that there

TABLE V

CATALASE ACTIVITY OF SEEDS TREATED 95 DAYS WITH $HgCl_2$ SOLUTIONS AND PERCENTAGE OF GERMINATION AFTER 6 MONTHS

CONCENTRATION OF $HgCl_2$ USED	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
N/800	0.5	2.3	2.9
N/1600	1.6	3.0	3.4
N/3200	1.3	3.1	3.9
N/6400	2.5	3.5	4.0
N/12800	3.0	4.0	4.6
N/25600	3.0	4.5	5.0
N/51200	3.2	5.0	5.9
N/102400	2.9	5.5	6.2
Water (check)	2.8	5.5	6.0

TABLE VI

EFFECT OF ETHER ON CATALASE ACTIVITY OF JUNIPER SEEDS KEPT AT 25° C.

AMOUNT OF ETHER PER 9 LITERS OF AIR	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
1.4 cc	2.9	6.0	6.1
2.8 cc	3.5	7.8	8.4
5.6 cc	4.8	10.0	10.9
8.4 cc	5.0	10.5	11.0
16.8 cc	5.6	11.0	11.5
Without ether (check)	2.8	5.5	6.0

was no germination. It is suggested, therefore, that one may have an enormous increase of catalase activity without a corresponding after-ripening of the dormant embryo.

To study the effect of oxygen, seeds were sterilized and exposed to air containing the following percentages of oxygen. Table VII gives the catalase activity for seeds at the end of 45 days. With the higher percentage of oxygen there was an increase of catalase

activity. At the forty-fifth day the remaining seeds were exposed to atmospheric air. Table VIII gives the catalase activity for the same lot of seeds at the end of 95 days, 50 days after replacing the oxygen by air. The point of interest here is the fall in catalase

TABLE VII

EFFECT OF OXYGEN ON CATALASE ACTIVITY OF SEEDS STORED 45 DAYS AT 25° C.

PERCENTAGE OF OXYGEN	LOT	OXYGEN IN CC. LIBERATED DURING		
		1 min.	5 min.	10 min.
30	1	2.5	4.4	4.8
55	2	3.4	5.3	5.6
80	3	3.7	6.2	6.6
100	4	4.8	7.5	8.8
Air (check)		3.8	6.2	6.9

TABLE VIII

REDUCED CATALASE ACTIVITY IN OXYGEN TREATED SEEDS WITH DECREASE IN PERCENTAGE OF OXYGEN

LOT	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
1	2.8	5.0	6.0
2	2.9	5.1	6.1
3	2.7	5.0	5.8
4	2.8	5.0	5.6
Air (check)	2.8	5.5	6.0

activity, at the ninety-fifth day, for the seeds that were exposed to 100 per cent O_2 during the first 45 days. None of these seeds germinated.

TEMPERATURE.—No other condition affected the development of the juniper seeds to the extent that temperature did. Both alternating and constant temperatures ranging from 15–30° C. were found to reduce the catalase activity and inhibit germination. Seeds exposed to winter weather (in soil and on moist filter paper) gave about 1 per cent germination. Those subjected to a temperature of 10–12° C. in running water showed a steady increase of catalase activity up to the time of germination. Between the

fourth and sixth month the germination reached 10 per cent, a very marked increase over that obtained at the higher temperatures. When the temperature of the water rose much above 12° C. germination ceased. These results show that the increased germination was not due to the removal of inhibiting substances from the coat, but to the effect of the low constant temperature.

Although many observers (**11, 20, 29, 30**) have reported a forcing action for freezing and freezing with thawing, these results show that when freezing really occurs it is very injurious. On March 14, 1919, 1000 air-dry seeds and 1000 moist seeds were placed at a constant temperature of −23° C.; and 1000 air-dry and 1000 moist seeds were subjected to an alternation of temperature between −23° and 10° C.. The latter seeds were left at each temperature for one week. After 45, 95, and 150 days of exposure samples were removed for study. The catalase activity of these seeds for 45 and 95 days is given in table IX. The catalase activity of seeds stored dry at −23° C. equaled that of untreated seeds, while that of seeds stored wet at −23° C. and wet or dry at the alternating temperature showed a marked decrease. There was no change in the oxidase or peroxidase activity. The seeds stored dry at −23° C. showed no increase of H^+ ion or titratable acid over that of the untreated seed. All other seeds showed a slight increase of sugar content and of H^+ ion concentration; also a 40 per cent gain of titratable acid. Both embryo and endosperm of these seeds, stored at the alternating temperatures, had the same H^+ ion concentration. The fats in these seeds were very soluble, not characteristic, and diffused throughout the endosperm and embryo. This general diffusion of the fats and the equal H^+ ion concentration for the embryo and endosperm indicate that the membranes had become more permeable by freezing (**16**). On staining these seeds with methylene blue they appeared to be dead. Samples of all seeds were put under favorable conditions for after-ripening and germination, but only the seeds that were stored dry at −23° C. after-ripened and germinated. These results prove that these low temperatures are very injurious unless the seeds are dry. It is probable that seeds stored at this low constant temperature and protected from moisture would retain their viability many years.

The alternation of temperature between −23° and 10° C. kills moist *Juniperus* seeds very soon, and in no sense can be looked upon as a forcing agent.

One lot of moist seeds was subjected to a temperature of −5° C., and a second lot to an alternating temperature of −5 and 5° C. The latter was exposed to each temperature for one week

TABLE IX

EFFECT OF TEMPERATURE ON CATALASE ACTIVITY OF JUNIPER SEEDS (NO. 10)

Material and treatment			O_2 in cc. liberated during			Total loss or gain over that of dry seeds (cc.)
Condition	Weight in gm.	Temperature °C.	1 min.	5 min.	10 min.	
	Stored 45 days					
Dry	0.025	.....	2.6	5.4	5.8	.....
Dry	0.025	−23	2.5	5.4	5.7	−0.1
Wet	0.026	−23	2.4	5.0	5.5	−0.3
Dry	0.023	−23 and +10*	2.2	4.9	5.4	−0.4
Wet	0.026	−23 and +10*	1.6	3.2	3.6	−2.2
Wet	0.025	− 5	3.0	5.1	5.8	0.0
Wet	0.025	0	3.0	6.0	6.5	+0.7
Wet	0.026	− 5 and + 5*	3.5	6.8	7.0	+1.2
Wet	0.026	+ 5	3.6	7.5	9.1	+3.3
Wet	0.026	+10	3.5	6.6	7.0	+1.2
Wet	0.026	+25	3.0	6.2	6.9	+1.1
	Stored 95 days					
Dry	0.023	−23	2.3	5.5	5.8	0.0
Wet	0.023	−23	2.4	5.1	5.7	−0.1
Dry	0.026	−23 and +10*	2.0	4.2	5.0	−0.8
Wet	0.025	−23 and +10*	1.0	2.8	3.1	−2.7
Wet	0.025	− 5	3.0	6.4	6.5	+0.7
Wet	0.025	0	3.4	7.0	8.0	+2.2
Wet	0.026	− 5 and + 5*	3.8	8.0	9.0	+3.2
Wet	0.026	+ 5	5.0	9.1	12.0	+6.2
Wet	0.026	+10	3.9	8.2	9.2	+3.4
Wet	0.026	+25	2.8	5.5	6.0	+0.2

*Weekly alternated between the two temperatures.

at a time. Table IX shows the catalase activity of these seeds for 45 and 95 days exposure. The lot stored at −5° C. showed scarcely any increase of catalase activity, while the lot exposed to the alternating temperature was more active. Both lots appeared morphologically and physiologically in good condition. There was a slight accumulation of sugar in all seeds. The first showed

no germination, but some of the latter germinated after about 6 months. While exposure to $-5°$ C. was not sufficient to injure the ungerminated seeds, it proved fatal to the germinated seeds. This is due to the fact that when the coat splits open the endosperm and embryo just doubles its water content and thereby dilutes the cell sap to a degree which permits ice crystals to form. Seeds at this period and later periods of development were killed by exposure to $-5°$ C. for seven days or less. The after-ripening and gain in catalase activity was a little more than enough to account for the sum of the effect at 5° C. These results show that the alternation of temperature between $-5°$ and 5° C. had slight forcing action. This forcing action is equal to that obtained by keeping seeds in running water at 10° C. It is also evident that seeds ready to germinate should not be subjected to $-5°$ C.

The early changes taking place in seeds put to germinate at $0 \pm 1°$ C. were similar to those at 5° C. except for being retarded. At this temperature the increase in catalase activity was very much retarded, although it was over 3 times that gained by seeds stored at $-5°$ C. per unit time. These seeds were studied as to storage material, H^+ ion concentration, and permeability, and found in good condition. The *Juniperus* seeds not only after-ripened but germinated at $0 \pm 1°$ C., even though it required 5 or 6 months.

Moist seeds were placed at 5° C. for germination. At this temperature the catalase activity increased most rapidly. The physiological changes occurring in the seed at 5° C. were most rapid, and will be discussed in detail under changes preparatory to germination. This constant temperature of 5° C. also gave rise to by far the largest percentage of germination, and the most vigorous seedlings.

It is evident from these germination experiments that: (1) temperatures above 10° C. and below 0° C. are not favorable for after-ripening and germination; (2) no one of the forcing agents as used was of value in germination; (3) the inclosing structures do not inhibit germination; (4) but the inhibiting conditions are to be found in the endosperm and embryo. These facts indicate that the juniper seed has a dormant embryo that must go through

a series of fundamental changes before germination. Of the many points of attack that are suggested by these experiments two were chosen: (1) changes preparatory to germination, and (2) means of shortening the after-ripening period.

Changes preparatory to germination

These are the changes that occur in the seeds stored at 5° C. which prepare them for germination. As the embryo of the dry seed is morphologically complete, increases very little in size, and shows only the transformation of cell contents, these processes could be spoken of as "foregermination"; but as this term has not been used in this country these processes will be referred to as after-ripening. The first point studied was the imbibition of water.

TABLE X

SHOWING PERCENTAGE ABSORPTION OF WATER
(SEEDS DRIED AT 105° C. FOR MOISTURE DETERMINATION)

Material	Percentage water	Weight	Weight after submergence for hours indicated									Percentage water at maximum imbibition
			2	4	8	16	24	72	96	120	360	
Entire seed	7.00	2.0018	2.16	2.37	2.40	2.41	2.42	2.42	2.43	2.42	2.39	23.22
Endosperm and embryo coats off during imbibition	7.19	0.290	.....	.....	.....	.....	0.37	0.38	.....	0.37	.....	28.94
Endosperm and embryo coats on during imbibition	7.19	0.291	.....	.....	.....	.....	.....	.....	0.375	.....	.....	24.84

Table X shows that the seeds decreased slightly in weight after a few days, even when submerged in water. In examining the tables given by LAKON for the water absorption of seeds of *Pinus*, it was noted that he incidentally obtained similar results. To follow this more closely, seeds with coats on were placed on moist filter paper at 5° C., and at times samples were selected, coats removed, and the percentage of water in the seed, exclusive of coat, determined. Table XI gives these results and the percentage of water in the seedlings as well. It should be noted that the water content of the seed decreased gradually until germination, when there appeared a very marked increase up to the time of the developed seedling. This percentage of water seems to be related to the change in the water absorbing power of seed contents, and

not to changes in the permeability of the coat, as later experiments show.

Table XII gives the changes of H^+ ion concentration as P_H values for the endosperm and different parts of the embryo during storage at 5° C. The outer cells of the embryo and its hypocotyl were the first parts to show an increased H^+ ion concentration.

TABLE XI

PERCENTAGE OF WATER IN SEEDS TAKEN FROM COATS AFTER DIFFERENT PERIODS OF EXPOSURE TO 5° C.

Seeds	Percentage water
Dry	7.19
After 5 days at 5° C.	24.84
After 15 days at 5° C.	23.34
After 30 days at 5° C.	23.09
After 60 days at 5° C.	23.00
After 90 days at 5° C.	23.21
After 100 days at 5° C. (coat splitting open)	52.64
After 130 days at 5° C. (seedlings 25 mm. long)	88.38

TABLE XII

H^+ ION CONCENTRATION OF SEEDS DURING AFTER-RIPENING*

Condition	Part of seed	P_H
Dry	Endosperm	4.4–6.0
Dry	Embryo	8.4–8.8
After 30 days at 5° C	Endosperm	4.6–5.2
After 30 days at 5° C.	Embryo	6.8–7.6
After 60 days at 5° C.	Endosperm	4.4–6.0
After 60 days at 5° C.	Embryo	6.8–7.6
After 90 days at 5° C.	Embryo hypocotyl	6.0–6.8
After 90 days at 5° C.	Endosperm	4.4–5.2
After 90 days at 5° C.	Embryo outer cells and hypocotyl	4.4–5.2
After 90 days at 5° C.	Embryo inner cells	4.6–6.0

* These determinations were made with the Clark and Lubs indicators.

The embryo showed a marked increase of H^+ ion concentration during after-ripening, while the endosperm with P_H value of 4.4 (concentration of H^+ ions $\times N = 0.72 \times 10^{-4}$), being already acid, showed very little change. This may indicate that the embryo is the principal seat of dormancy. Table XIII gives the increase of titratable acid in the endosperm and embryo during after-ripening. The increased acid was determined by titrating with N/50 NaOH,

using phenolphthalein as the indicator. To show that this increased acid content is real the calculated dry weight of the seed material used is given.

The fat of the dry seeds is stored as very large globules, contrary to the statements of CZAPEK, which divide and become continually smaller as after-ripening goes on. Just preceding germination these fat globules, in the active growing cells, become reduced to microscopic size, although CZAPEK states that the microscopically divided fat of dry seeds collects into globules with early

TABLE XIII

INCREASE OF TITRATABLE ACID IN ENDOSPERM AND EMBRYO

Condition	No.	Dry weight in gm.	N/50 NaOH in cc.	Increased acid per unit volume of water
Dry	80	0.165	0.56	.....
Dry	160	0.348	1.25	.....
Dry	80	0.160	0.53	.....
Dry	80	0.164	0.55	0.0254
After 15 days at 5° C.	80	0.163	0.57	0.0989
After 30 days at 5° C.	80	0.172	0.58	.....
After 30 days at 5° C.	80	0.172	0.60	0.0866
After 60 days at 5° C.	80	0.169	0.70	.....
After 60 days at 5° C.	80	0.167	0.70	0.0685
After 95 days at 5° C.	80	0.157	0.85	.....
After 95 days at 5° C.	80	0.163	0.90	0.0564
Open seeds	80	0.189	1.50	0.1426
Hypocotyl 2 mm	80	0.195	2.60	0.1615
Seedlings	80	0.210	8.00	.....
Seedlings	80	0.204	8.00	0.1865

growth (7). This dispersion of the fatty material brings clearly into play surface tension, adsorption power, and many other forces resulting from the great increase of specific surface. Such a dispersion could lead to a more rapid digestion of the fats, thus materially aiding the transformation of fats to carbohydrates and the accumulation of energy for germination. The importance of making the fats more capable of transformation to carbohydrates should not be overlooked. It is also probable that this dispersion reaches a degree of division where it could aid in the translocation of fats as such. Thus highly dispersed fatty material would be carried through the cell walls at points of protoplasmic connection.

Early during the process of after-ripening there was a slight decrease in the fat content of the endosperm cells surrounding the embryo. The most rapid disappearance of fat occurred in the hypocotyl end of the endosperm at approximately the ninety-fifth day. This rapid decrease of fat was accompanied by an increase in the sugar content of the adjoining hypocotyl cells. This was the first noticeable increase of sugar during after-ripening. At this time the coat splits open, probably partly due to the increased osmotic pressure of the newly synthesized sugar. With these changes the first detectable starch was found. It increased very rapidly in these cells, until they seemed to be completely packed. Traces of starch appeared in the cotyledons and they soon became green, a point to be taken up later. Thus during the preparation for germination the stored fat was transformed into carbohydrates. Not all the fat is changed directly into carbohydrates. Under certain conditions it seems to be changed into forms more capable of translocation and used to synthesize other compounds, or even stored again. It seems that a large part of the food material of these seeds during after-ripening, germination, and the development of the seedling is translocated in this form.

Amino acids appear in both ungerminated (dry) and germinated seeds. Table XIV gives the amino acids found in these seeds, as well as a rough estimate of their quantities. The histidine in the endosperm was used up completely during the after-ripening.

Table XV gives the changes occurring in the proteins of *Juniperus* seeds during germination as indicated by color reaction. These results show that soluble proteins increased during after-ripening. It was also shown that the proteins were hydrolyzed during after-ripening by the determination of amino nitrogen and the formal titration. Table XVI gives the results of the VAN SLYKE determination for amino acids. This table shows that the 5 minute reaction period was too short, which indicates the presence of amino acids with other than α-amino groups. The arginine found would account for the increase under 30 minutes reaction. These figures prove that there was a marked hydrolysis of the proteins during after-ripening, as well as during germination and the development of

seedlings. As this protein digestion goes on, the number of free amino groups increases because of the splitting amino-carboxyl linkings. When hydrogen of the free amino group is replaced by

TABLE XIV

TESTS FOR AMINO ACIDS IN JUNIPER SEEDS

AMINO ACIDS	CRYSTALLIZATION	COLOR REACTIONS	AMOUNT OF AMINO ACIDS IN			
			Dry seeds		After-ripened seeds	
			Endosperm	Embryo	Endosperm	Embryo
Histidine...	+	Ehrlich's diazo	+++	+++	+	++
Tyrosine....	+	Ehrlich's diazo	+	+	++	++
Tyrosine....	+	Millons	+	+	++	++
Tyrosine....	+	Xanthroproteic	+	+	++	++
Cystine....	+	Sulphur reduction	+	+	+	+
Leucine....	+	.....	+	+	++	++
Arginine....	+	.....	+	+	++	++

TABLE XV

CHANGES IN STORED PROTEIN FOOD DURING GERMINATION

REACTIONS	DRY SEEDS			AFTER-RIPENED SEEDS		
	Endosperm	Embryo	Hypocotyl	Endosperm	Embryo	Hypocotyl
Biuret.....	++	+++	+++	+	+	++
Millons.....	+++	+++	+++	++	++	+
Xanthroproteic.....	+++	++	++	+++	++	+
Berlin blue.....	+	?	?	+	++	+++

TABLE XVI

INCREASE OF AMINO NITROGEN DURING AFTER-RIPENING AND GERMINATION

Condition of seed material	Time of reaction (min.)	N (cc.)	Temperature	Pressure (mm.)	Nitrogen obtained (mg.)	Amino acid as percentage of dry weight
Dry or resting.....	5	0.25	23.2	753.2	0.138	0.035
Coats bursted after 100 days at 5° C.....	5	0.50	24.7	750.8	0.274	0.270
Hypocotyl 3 mm. long or after 105 days at 5° C.....	5	0.59	24.5	751.0	0.324	0.275
Developed seedling or after 130 days at 5° C.....	5	1.41	24.5	751.3	0.775	0.921
Dry or resting.....	30	0.60	23.2	753.2	0.332	0.036
Coats bursted after 100 days at 5° C.....	30	0.84	24.2	750.7	0.462	0.279
Hypocotyl 3 mm. long or after 105 days at 5° C.....	30	1.17	24.5	750.7	0.642	0.280
Developed seedling or after 130 days at 5° C.....	30	1.97	25.2	750.7	1.078	0.935

methylene, the basicity becomes reduced; and the substituted acid can then be titrated with sodium hydrate as a measure of protein hydrolysis. Titrations made on a second lot of seeds according to the SORENSEN method gave results similar to the VAN SLYKE determinations.

The growth in these seeds occurring before germination is very meager. There is no morphological change in endosperm or embryo, although the latter increased slightly in length. After the appearance of sugar the hypocotyl exerts a forward pressure, separating the sides of the swelling cap which forces the coat open. At this moment the cap is under so much pressure that it is distorted, and a sharp angle is formed between its end and sides. The growth following this stage will be discussed later.

TABLE XVII

RESPIRATION OF SEEDS AT DIFFERENT PERIODS OF DEVELOPMENT AT 25° C. (7 CC. VOLUME)

Condition of seeds	No.	Green weight	Days	Percentage CO_2	Percentage CO_2+O_2	Percentage O_2 used	$\frac{CO_2}{O_2}$	Mgm. CO_2 per hour per gm.	Mgm. O_2 per hour per gm.
Dry.....	500	1.250	5	1.18	20.82	1.57	0.76	0.00098	0.0011
After 5 days at 5° C.	50	0.125	1	3.15	20.39	3.77	0.84	0.1311	0.1347
After 30 days at 5° C.	10	0.030	3	3.78	20.82	3.98	0.94	0.218	0.1976
After 60 days at 5° C.	10	0.027	3	3.80	20.70	3.90	0.97	0.2352	0.2151
After 90 days at 5° C.	10	0.028	3	3.80	20.68	3.90	0.97	0.2354	0.2075
After 100 days at 5° C.	10	0.028	3	4.10	20.00	6.00	0.68	0.2486	0.3192
After 130 days at 5° C.	10	0.099	1	9.30	20.56	9.74	0.95	0.4890	0.4398

Table XVII gives the results of the respiration experiments obtained by the use of the Bonnier and Mangin apparatus. There was a great increase in the respiratory intensity during the first 5 days and after the seeds split open. These are the periods when the seed increased in water content. There was a very slow increase in the respiratory intensity during after-ripening, even though the water content decreased. The respiration quotient increased very slightly during after-ripening, but decreased to a minimum at germination. Not only does this low respiratory quotient of 0.68 indicate the time of intense fat metabolism, but at this particular period it was found that the fats were being transformed into carbohydrates. It would be interesting to know this quotient at 5° C., as it would probably be much lower. After germination

the seedlings gradually attained the ratio 1:1. This rise in the respiratory quotient was probably due to the oxidation of carbohydrates and the more intense respiration of the seedlings.

Table XVIII gives the results of intramolecular respiration. The method used was that of NICOLAS (**26**). The point to be noted here is the low 1/N ratio (the intramolecular or anaerobic respiration divided by the normal respiration) for the seedlings.

Peroxidase was more generally present than oxidase. Quantitative oxidase activity determinations were made with the Bunzel apparatus. These results showed that there was no appreciable increase of oxidase activity until after germination.

TABLE XVIII

INTRAMOLECULAR RESPIRATION OF JUNIPER SEEDS, NO. 10, AT 25° C. (7 CC. VOLUME)

Condition of seeds	Weight	Days	Percentage CO_2	1/N ratio
After 30 days at 5° C.....	0.030	3	1.70	0.44
After 90 days at 5° C.....	0.028	3	1.67	0.43
After 100 days at 5° C.....	0.028	3	1.66	0.40
After 130 days at 5° C.....	0.099	1	0.95	0.10

The results of catalase determinations are given in table IX, which gives the average of a great number of experiments. It was found that (1) when seeds were placed under ordinary germination conditions at 5° C. the increase of catalase activity gave a measure of the after-ripening; (2) the gain in catalase activity above that of air-dry seeds was greatest at 5° C. in a germinator; (3) the gain at the other temperatures was slow at best; and (4) seeds soon lose their catalase activity when in a germinator at temperatures above 25° C. The precautions used in the catalase determinations have been stated.

CROCKER (**6**) speaks of the rise in vigor of seeds, as shown by their resistance to fungal attack, during after-ripening. The juniper seed is protected against fungi before germination by the heavy lignin coat. It was found that juniper seeds which had not been after-ripened soon succumbed to fungal growths with the removal of the coats. After-ripened juniper seeds, however, when freed from the coats, withstood dense fungal growths.

Many such experiments indicate that the vigor and resistance of the seed to fungi increased greatly during the after-ripening process. These results prove that the juniper seed has a dormant embryo that goes through certain definite and well defined fundamental chemical and physical changes before germination can occur. Some changes occur also in the endosperm.

SHORTENING AFTER-RIPENING PERIOD AT 5° C.—The after-ripening period was shortened considerably by the constant temperature of 5° C., as has been shown, but attempts to shorten further this after-ripening period at 5° C. seemed to meet with difficulties. GUPPY'S (**15**) method of forcing seeds to germinate by placing the soft pre-resting seeds (caught before going into the rest period) at 20° C. was tested. None of these seeds germinated, and it is evident that the juniper seed must pass through a more or less definite rest and after-ripening period. This period was not shortened by the removal of the seed coats. ECKERSON (**10**) states that dilute acids greatly shorten the after-ripening period of the hawthorn. Dilutions of HCl between N/100 and N/3200 had no effect upon the juniper seed. Neither sugar, enzyme, nor vitamine solutions shortened this period. Hydrogen peroxide gave no results. In the treatment with different percentages of oxygen, it was found that the catalase activity increased slightly with increased oxygen pressure, and that the germination was retarded two months. Seeds were treated with different percentages of ether ranging from 0.002 to 6.000. As long as these seeds were under the influence of ether they showed a decrease in catalase activity proportional to the percentage of ether used. After atmospheric conditions were restored, all seeds recovered their catalase activity, but the after-ripening period was lengthened from 1 to 3 months depending on the low and higher percentages of ether. If the ether acted by decreasing the permeability, then it was evidently reversible, contrary to the work of OSTERHOUT (**27**). It is more probable, however, that the ether acted as a narcotic agent. This is also shown by the behavior of the seed. Carbon dioxide was used in concentrations ranging from 0.5 to 100 per cent with a six day exposure. The higher percentages increased the catalase activity and shortened slightly the after-ripening period. The action here

was probably due to increased acidulation in the presence of an abundance of CO_2 and H_2O which could favor the digestion of fats and germination (**25**). Desiccation and moistening again of seeds at about the forty-fifth day after being placed in the germinator shortens the after-ripening period from 5 to 10 days. This may be due to one of the following causes: (1) earlier after-ripening of the coats, as they are found to split off more readily when desiccated, (2) upsetting of the chemical equilibrium by the great extraction of water, or (3) the increase of H^+ ion concentrations.

Discussion

CATALASE.—The catalase activity, as has been noted by previous investigators (**1, 6**), was found to bear some relation to respiration. Increased catalase activity accompanied the intense respiration of *Juniperus* seeds stored in high percentages of oxygen, as decreased

TABLE XIX

INCREASED CATALASE ACTIVITY WITH DEVELOPMENT (FIGURED PER UNIT DRY WEIGHT)

CONDITION OF SEEDS	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
Air dry	2.5	5.4	5.8
After 45 days at 5° C.	3.6	7.5	9.1
After 95 days at 5° C.	5.0	9.1	12.0
After 100 days at 5° C. (coats split)	5.3	12.2	14.4
After 130 days at 5° C. (seedlings)	10.5	23.1	28.5

catalase activity accompanied the low respiration of seeds stored in low percentages of oxgyen. With the intense respiration at high temperatures there was an increased catalase activity, even though the seeds did not after-ripen or germinate. The highest catalase activity and the most intense respiration per unit of dry weight was found in the seedling stage (cf. tables XIX and XVII). The desiccation of seeds to a slight extent, which makes for a rapid absorption of oxygen through the coat, increased the catalase activity. Desiccation to the extent of retarding respiration reduced the catalase activity. Table XX shows these results. Both the respiration and the catalase activity of seeds were reduced at will

by submerging them in water. Although increased catalase activity generally accompanied intense respiration, this relationship did not always hold, for when seeds were submerged a long time the catalase activity slowly increased, but there was no increase of respiration intensity. An examination of tables IX and XVII will show that the catalase gain was proportionally very much larger than the respiration gain during after-ripening. It will also be noted that the catalase gain was greatest at 5° C., where the respiration was low. It is evident, therefore, that there may be increased catalase activity without an increase of respiration.

TABLE XX

CATALASE ACTIVITY OF AFTER-RIPENED AND DESICCATED SEEDS, NO. 30 (CALCULATED DRY WEIGHT 0.0696)

Treatment	O_2 cc. liberated after 10 min.
Complete imbibition	33
Slight desiccation	38
Strong desiccation	32
Second imbibition	36

RATE AND PERCENTAGE OF GERMINATION.—Juniper seeds germinate most readily at the low temperature of 5° C. These seeds germinate, although very slowly, at 0±1° C. They also germinate at 10° C. Seeds after-ripened at 5° C. and then placed at 10° C. germinated slower than those left at 5° C. After-ripened seeds were thrown into a state of secondary dormancy by exposure to temperatures above 12° C. Their catalase activity gradually decreased and germination ceased. After being thrown into secondary dormancy, several weeks at 5° C. were required to after-ripen the seeds again. The seeds which sank in water gave between 75 and 80 per cent germination at 5° C.

GROWTH OF SEEDLING.—Table XXI gives the rate and extent of growth for seedlings exposed to the light or the dark at different temperatures. All seeds were germinated at 5° C. and then transferred to the different temperatures. The length of the extending hypocotyl at the time of transfer was 0–1 mm. The seedlings grew the longest and fastest at 25° C. At 30° C. they never attained

a normal length, while at 0± 1° C. there was a slow but definite growth. It is important to note that 15° C. seedlings developed first and appeared the most healthy and sturdy. These seedlings

TABLE XXI

EFFECT OF LIGHT AND TEMPERATURE ON RATE AND EXTENT OF GROWTH*

TEMPERATURE	LIGHT	LENGTH OF HYPOCOTYL IN MM. FROM TIME OF TRANSFER					
		3 days	7 days	11 days	13 days	18 days	26 days
30° C...	Dark	1	5	7	12	18	20
25° C...	Dark	10	35	40	55	60	Seedling
15° C...	Dark	4	18	29	Seedling	.....	.....
10° C...	Dark	3	10	30	35	Seedling	.....
10° C...	Light	3	11	29	30	Seedling	.....
5° C...	Dark	2.5	4	12	15	26	35
0± 1° C...	Dark	0	1	2	3	4	5
− 5° C...	Dark	0	Killed	0	0	0	0

*Average of 50 trials.

at 15° C. also showed the earliest and greatest development of chlorophyll. Light did not seem to affect unusually the extent or rate of growth.

PIGMENTS.—Carbohydrates and temperature may condition chlorophyll development. The seedling was found to develop chlorophyll in total darkness. Thus the cotyledons become green long before they break out of the coat. Chlorophyll appeared first in the cotyledons and accompanied the formation of starch. This points to the conclusion that soluble carbohydrates are necessary for the formation of chlorophyll, the view advanced by PALLADIN (**28**). Table XXII gives the results of experiments planned to determine the effect of light and temperature on greening. This shows that light affects in no way the rate or apparent depth of greening. It also shows that at 30° C. and at 0± 1° C. chlorophyll did not develop. As the plastids were found to be in good condition, it was thought probable that a lack of building material was inhibiting chlorophyll development. Glucose cultures were made, therefore, but the seedlings again failed to develop chlorophyll. This indicates that a certain temperature is necessary for chlorophyll development, regardless of carbohydrate supply,

the maximum, optimum, and minimum temperatures for chlorophyll formation in the seedlings being represented by temperatures somewhat below 30, 15, and somewhat above 0° C.

Seedlings grown at 0± 1° C. developed anthocyanin, while those grown at 30° C. developed xanthophyll. When cultures at 0± 1° C. were supplied with glucose they developed more anthocyanin. The seedlings grown at 30° C. were made to develop anthocyanin by the addition of glucose. From the foregoing it appears that the seedlings form various pigments according to their reserve sugar

TABLE XXII

EFFECT OF LIGHT AND TEMPERATURE ON DEVELOPMENT OF CHLOROPHYLL*

TEMPERATURE	LIGHT	ESTIMATED PERCENTAGE OF COLOR AFTER TRANSFER								
		1 day	4 days	6 days	8 days	11 days	13 days	18 days	26 days	50 days
30° C.	Light	0	0	0	0	0	0	0	0	0
25° C.	Light	5	25	50	50	50	65	.....	.....	.....
15° C.	Light	5	25	50	75	75	100	.....	.....	.....
10° C.	Light	5	25	50	75	.....	.....	100	.....	.....
10° C.	Dark	5	25	50	75	.....	.....	100	.....	.....
5° C.	Light	.....	.....	.....	5	.....	.....	50	.....	.....
5° C.	Dark	.....	.....	.....	5	.....	.....	50	.....	.....
0± 1° C.	Light	.....	.....	.....	.....	.....	.....	.....	.....	0
0± 1° C.	Dark	.....	.....	.....	.....	.....	.....	.....	.....	0

*Average of 50 trials.

supply. Seedlings with little sugar tend to develop xanthophyll, those with more sugar chlorophyll, and those with an abundance of sugar anthocyanin.

PRACTICAL APPLICATION.—The foregoing experiments make it possible to devise an outline for the practical production of juniper plants. This should be of interest to growers, since it has furnished a means of increasing many fold the percentage of germination and of developed seedlings. After collection, the seeds are freed from the berries, sorted, and sterilized as has been described. The seeds are then put into Petri dishes or covered flat vessels on filter paper supported by wet cotton. These vessels of seeds are kept at a constant temperature of about 5° C. (41° F.) for after-ripening, which takes about 100 days. This after-ripening period can be shortened 10 days by drying slightly and moistening again the seeds at about the forty-fifth day. When the coats have split

open and the hypocotyls are $\frac{1}{8}$ in. long, the seedlings are transferred to pans or beds of leaf mold and sand kept at 15° C. (60° F.). In no case should ungerminated seeds (seeds that have not split open and developed a short hypocotyl) be transferred from the germinator at 5° C. (41° F.). The germinated seeds, after being transferred to beds or pans, should be protected by glass plates and paper for the first few days.

Although these seeds have been germinating during every month of the year, advantage can be taken of the temperature conditions by placing them in the germinator about January. The importance of this after-ripening and germination at 5° C. cannot be overemphasized.

Summary

1. The germination of non-after-ripened juniper seeds under ordinary conditions is very low, amounting to 1 per cent.

2. These seeds are protected by a semipermeable and thick coat which makes up 75 per cent by weight of the entire seed. Acids enter very slowly, while bases, silver and mercury salts, enter rapidly. While the coat serves as a protection against fungal attack and prevents water-imbibed seeds from expanding and rupturing the tissues before after-ripening is accomplished, it takes little or no part in the dormancy or after-ripening of the seed.

3. Food material in the resting seed is stored in the form of fats and proteins, with traces of glucose but no starch. The resting seed endosperm has a P_H value of about 5, while that of the embryo is about 8.

4. Although some forcing agents changed the respiration and catalase activity of seeds, it was not possible to force the germination of non-after-ripening juniper seeds by high temperature, alternating temperature, wounding, warm bath, dry air, removal of coats, treatment with hydrogen peroxide, mercuric chloride, ether, carbon dioxide, oxygen, light, soil, dilute acids, dilute bases, nitrates, suphates, or strong acids.

5. Freezing and thawing as such has no forcing action on the germination of juniper seeds, neither does it hasten after-ripening. Freezing and thawing produces marked chemical changes in this

seed, but these changes, as has been outlined, are quite different from those occurring during after-ripening. Seeds ready to germinate (after the coat is cracked and their water content increased to 52 per cent) are killed by an exposure to $-5°$ C.

6. The juniper seed has a dormant embryo that must after-ripen before germination. After-ripening occurs at temperatures between $0 \pm 1°$ C. and 10° C., although fastest at about 5° C.

7. The changes that accompany after-ripening of the juniper seed at 5° C. were found to be as follows: (1) rather rapid and complete imbibition, followed by a steady slow decrease in water content during after-ripening or until near germination; (2) increased H^+ ion concentration, especially of the embryo; (3) an increment of titratable acid; (4) a steady and enormous increase in the degree of dispersion of the stored fat; (5) decrease in the amount of stored fat and protein, with an increase of sugar content and the first appearance of starch; (6) the translocation of food in the form of fat or fatty acids from endosperm to embryo; (7) a seven-fold increase in the amino acid content, and a complete disappearance of histidine from the endosperm; (8) an increase of soluble proteins, with a marked hydrolysis of the stored proteins: (9) slight growth of embryo; (10) very slight increase of the respiration intensity; (11) increased respiratory quotient; (12) decreased intramolecular respiration; (13) a doubling of the catalase activity; and (14) the rise in vigor of seeds as shown by their resistance to fungal attack.

8. In conjunction with after-ripening at 5° C., desiccation seems to be the only promising means of shortening this after-ripening period.

9. The time at which the hypocotyl breaks through the nucellus was fixed as the end of after-ripening and the beginning of germination.

10. Neither the resting nor the after-ripened juniper seeds yield more than about 1 per cent germination at temperatures above 15° C. Seeds after-ripened at 5° C., then placed at 10° C., germinate slower than those left at 5° C. When after-ripened seeds are transferred from 5° C. to temperatures above 15° C. they are thrown into a state of secondary dormancy. Hence these seeds require a low temperature for germination as well as for after-ripening, and therefore no seed should be transferred to

higher temperatures until germination has started. If these seeds are given sufficient time they will germinate, even at 0 ± 1° C.

11. Subsequent to after-ripening and germination at 5° C., the best temperature for seedling development is 15° C.

12. The development of chlorophyll in the juniper seed and seedling was found to be independent of light, but conditioned by the temperature range. Seedlings grown at temperatures of 0 ± 1° C. or 30° C. never developed chlorophyll. Anthocyanin development in seedlings seems to depend upon relative temperature and carbohydrate supply.

13. A more complete chemical analysis of these seeds at different stages of development will be given in a later paper.

Acknowledgments are due Dr. WILLIAM CROCKER and Dr. S. H. ECKERSON, under whose direction and criticism this work was carried on.

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GAMETOPHYTE AND SEX ORGANS OF REBOULIA HEMISPHAERICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 276

ARTHUR W. HAUPT

(WITH TWENTY-ONE FIGURES)

The Marchantiales represent a very natural group of liverworts whose evolutionary tendencies are more obvious and striking than those of any other order of Bryophytes. The characters which distinguish them from the Jungermanniales are remarkably constant, and yet the structural changes which one meets in passing from the lower to the higher forms are represented by an almost complete series of intergrades. In order to determine the phylogenetic relationships of the little investigated genus *Reboulia*, the present study was undertaken.

According to SCHIFFNER (7), *Reboulia* comprises 2 species: one confined to Java, and the other, *R. hemisphaerica*, a polymorphic species, cosmopolitan in distribution. STEPHANI (9) recognizes only *R. hemisphaerica* as a single polymorphic species, including as synonyms several other forms which various authors have raised to specific rank. CH. and R. DOUIN (3) have described 2 new species from France and other parts of Europe which they have named *R. occidentalis* and *R. Charrieri*. These are distinguished from *R. hemisphaerica* chiefly on the basis of the size and wall markings of the mature spores, the size and number of lobes of the female receptacle, and the position and behavior of the male receptacle. The latter forms two groups which come to occupy marginal positions on the thallus instead of remaining undivided and median as in *R. hemisphaerica*. The writer has observed this in rare cases in *R. hemisphaerica*, and if the small size of certain parts be explained on the basis of impoverished vegetative conditions, there seems to be little justification for the establishment of these 2 new species.

SCHIFFNER (7) divides the Marchantiaceae into the 3 subfamilies, Corsinioideae, Targionioideae, and Marchantioideae.

Following LEITGEB (6), he further separates the Marchantioideae into the Astroporeae, Operculatae, and Compositae. CAVERS (2) has shown that the characters which separate these groups are not entirely constant; yet he recognizes their individuality, but elevates them to the rank of families and renames them Cleveaceae, Aytoniaceae, and Marchantiaceae. *Reboulia* belongs to the Operculatae of LEITGEB or to the Aytoniaceae of CAVERS.

Material

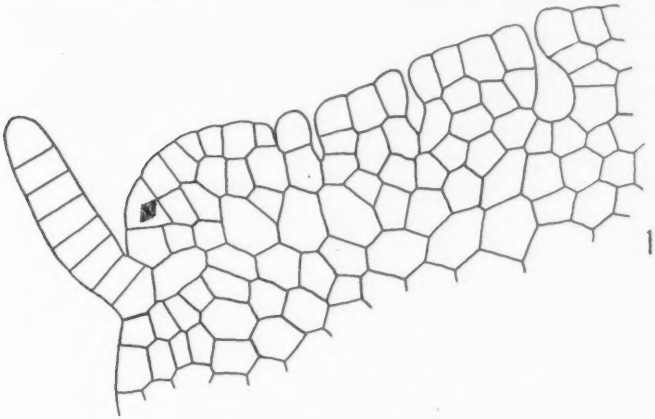
The writer is indebted to Dr. W. J. G. LAND for the material used in this investigation. Four collections were made by him over a period of 10 years during the months of September, October, and November at Rome, Indiana. A few of the slides were made by Mr. S. A. IVES, but most of them were prepared by the writer.

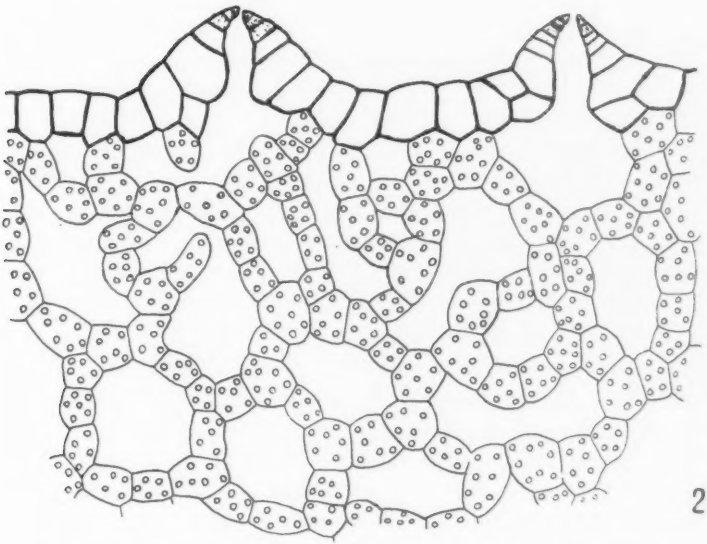
Thallus

The gametophyte plant body of *Reboulia hemisphaerica* is a pale green, dichotomously branched thallus with slightly undulate margins and a distinct midrib. Rhizoids and scales arise from the ventral surface. Both smooth and pegged rhizoids occur, their ends often being highly contorted when in contact with soil particles. The dark red ventral scales are 2-ranked and point diagonally forward and outward. They do not arise from the splitting of a single scale as in most species of *Riccia*, but are separate from the beginning. The scales are 1 cell thick except near their place of attachment to the thallus where they are often 2 cells thick. They are unappendaged.

The body of the thallus is differentiated into a lower colorless region of compact elongated cells with rather thick walls, and an upper region of loose chlorophyllose tissue containing large air chambers. Intracellular fungi live in the lower region (fig. 5). The walls of the epidermal cells are slightly thickened and are devoid of chloroplasts except near the growing point of the thallus (fig. 2). The plastids in the air chamber region are rather large and contain several starch grains with distinct hila. Plastids in the cells in the growing region are small and contain no starch. The development of the large starch-producing plastids was

studied, and a series obtained as illustrated by fig. 6 *a–d*. Small oil globules are very abundant in the apical cell region and occur less abundantly in the cells of the older parts of the thallus. The





FIGS. 1, 2.—Fig. 1, apical cell of thallus and young air chambers, ×470; fig. 2, upper region of thallus showing air chambers and pores, ×250.

formation of a definite oil cell from an ordinary vegetative cell is a common feature of the genus. No gemmae are produced.

The thallus grows by means of a single cuneate apical cell which cuts off segments from its four sides (fig. 1). The air chambers form a very extensive system of irregular air passages separated by thin partitions, but more or less connected (fig. 2). No chlorophyllose filaments are formed. LEITGEB (6) has observed that the secondary partitions arise from all sides of the primary air chambers and grow toward the center, and that the boundaries of the primary chambers are soon lost, resulting in the entire air chamber region becoming broken up into a uniform spongy tissue.

The most complete study of the development of the air chambers among the Marchantiales is that of BARNES and LAND (1). The situation in *Reboulia* is in agreement with the results of their investigations on other members of the group. The air chambers of *Reboulia* arise immediately behind the apical cell of the thallus by intercellular splittings which start at the surface of the thallus and progress inward (fig. 1), reaching the line of differentiation between the dorsal and ventral regions. Secondary splittings occur deep within the dorsal region, and do not reach the surface. No evidence was found to suggest centrifugal splitting in the region behind the apical cell, nor air chamber formation, as LEITGEB has described, by the upgrowth of adjacent cells of the thallus.

The cells forming the air pore margins are attenuate, and are developed by segmentation from the adjacent epidermal cells (fig. 2). This type of air pore also occurs on the male receptacle, but those on the female receptacle are barrel-shaped (fig. 4). The air chambers in the female receptacle develop like those of the main body of the thallus. CAVERS (2) has reported barrel-shaped air pores on the male receptacle of *Reboulia hemisphaerica*, but these were not present in the material used for this investigation.

Sex organs

The plants of *Reboulia* are monoecious, and the antheridia and archegonia are borne in separate median groups on the dorsal surface of the thallus. The antheridial group is only slightly raised above the general surface of the thallus, and the archegonial

receptacle becomes lifted up on a stalk only after the sporophytes are approaching maturity. The archegonia appear in the autumn, fertilization occurs, and the embryo develops immediately. The sporophyte, however, does not mature until the following spring.

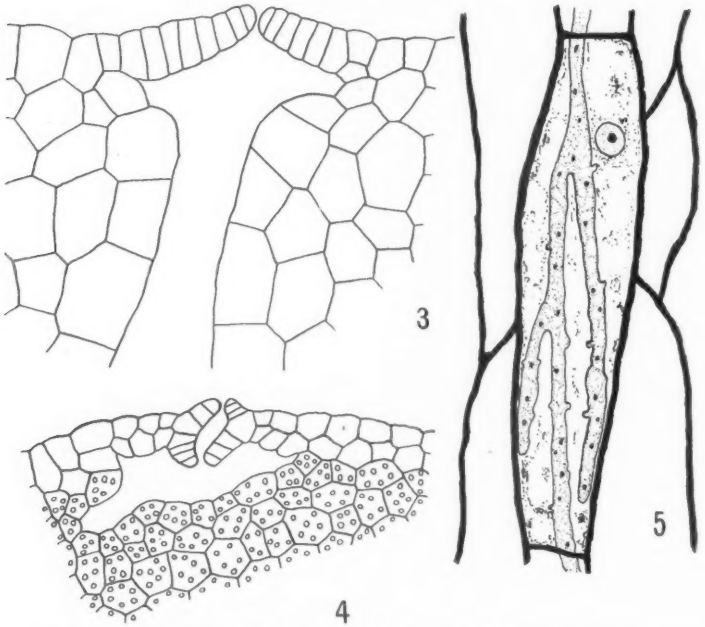
Antheridium

The antheridia occur in sessile lunate receptacles which are sometimes irregular in outline and broken up into several pieces. The receptacle is cushion-shaped and slightly raised above the general level of the thallus, but the antheridia themselves are rather deeply sunken in the main body of the thallus. Four, or occasionally 5, antheridia are usually seen in cross-section, and 4–6 in longitudinal section, but often more, and in rare cases as many as 24 were counted in longitudinal section. The number of antheridia in a receptacle, therefore, varies from about 16 to over 100.

Usually 2 and rarely 3 groups of antheridia are produced successively on the thallus before the appearance of the archegonial receptacle. They are formed in rather close proximity (about 7–10 cells removed) to the apical cell, and each group becomes isolated by a rather wide area of sterile tissue of the thallus. In all cases the antheridia develop strictly in acropetal succession from segments of the apical cell. Air chambers are abundantly formed in the raised portion of the thallus which communicate with the surface by means of air pores similar to those which occur on the rest of the thallus. By growth of the epidermal cells around the pores which communicate with the antheridial cavities, a plate of cells is formed containing a small central perforation through which the sperms escape (fig. 3).

The simplest arrangement of the antheridia among the Marchantioideae may be represented by *Clevea*, in which they are sunken in the back of the thallus and not arranged in groups. In *Sauteria* the antheridia occur in raised groups along the median line of the thallus. In *Fimbriaria* and *Reboulia* there is an intermittent development of antheridia, which occurs in raised groups without checking the activities of the apical cell. The situation is similar in *Aytonia* (*Plagiochasma*), except that the apical cell does not

function while the antheridial group is developing, but later continues the growth of the thallus. In *Dumortiera* the antheridial receptacle is lifted up on a short stalk and represents a definite branch system which terminates apical growth of the thallus. In *Marchantia* the situation is similar, but the stalk is long.

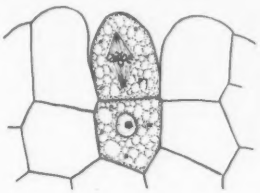


FIGS. 3–5.—Fig. 3, pore on male receptacle through which sperms escape, ×470; fig. 4, air chambers and pore in female receptacle, ×250; fig. 5, cell from ventral region showing intracellular fungus, ×470.

The development of the antheridia of *Reboulia* is similar to that of the other Marchantiales which have been investigated. The superficial papillate initial is soon overgrown by the adjacent cells of the thallus, so that the antheridium comes to lie in a pit; the development of the antheridial pit is therefore very different from that of the air chambers. The first division of the initial is transverse and separates the imbedded portion of the stalk from the rest of the antheridium (fig. 7). Additional transverse walls

appear in the outer cell without a definite sequence, resulting in a filament of 4 cells (fig. 8). Vertical walls are then formed in two planes at right angles to each other, which usually are first developed in the basal tiers, or sometimes elsewhere (fig. 9). After the formation of the first vertical walls in the lower tiers, additional transverse divisions usually occur below, and as a consequence 5 or 6 tiers of cells are formed (fig. 10).

In *Marchantia polymorpha* STRASBURGER (**10**) has reported that the 3 transverse walls in the outer cell appear in centrifugal succession, although he shows no mitotic figures to prove this. Fig. 8 suggests that this may occur in *Reboulia*. STRASBURGER has also shown that the vertical walls appear in the 4-celled stage, but that no additional transverse walls are formed until the wall



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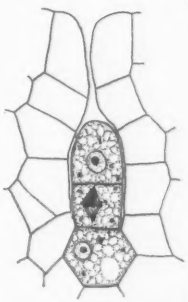

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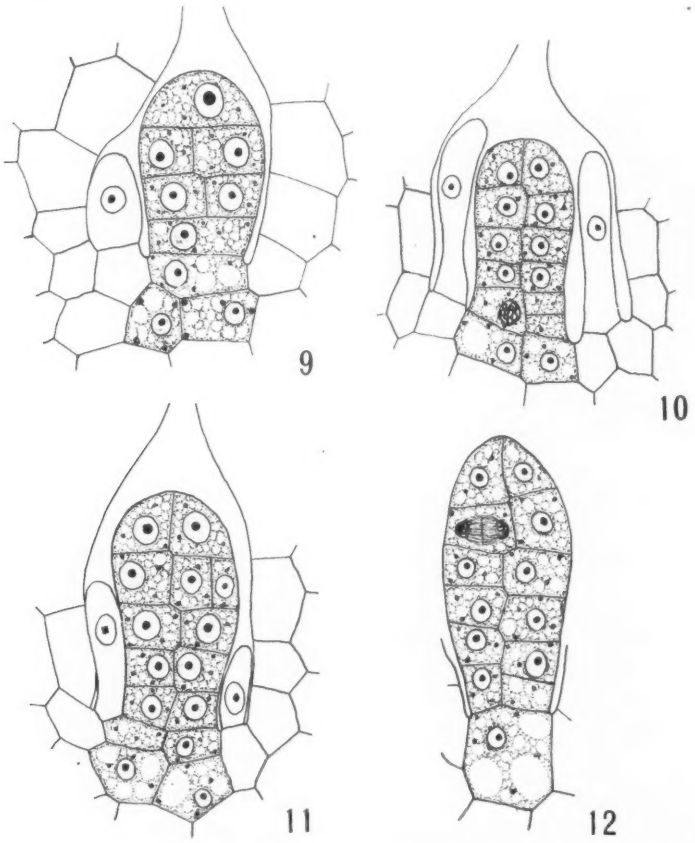
FIGS. 6–8.—Fig. 6, series showing development of starch grains in plastids, ×1850; fig. 7, first division of outer segment of antheridial initial, ×790; fig. 8, division of the lower segment of the outer cell, ×790.

and spermatogenous cells are differentiated. *Marchantia* therefore seems to be somewhat more advanced in this respect. DURAND (**4**), however, who also studied *M. polymorpha*, has found that additional transverse walls may follow the appearance of the vertical walls, but in no observed case did he find that they preceded the first vertical walls.

Periclinal divisions next occur invariably in the 3 uppermost tiers of cells (figs. 11–13), separating the inner spermatogenous cells from the outer sterile wall cells. The cells in which no periclinal appears form the stalk. The further development of the

spermatogenous tissue is like that of the other Marchantiales. The sperms differ in no way from those of the other members of the group.



FIGS. 9–12.—Older antheridia: fig. 9, vertical walls appearing in middle tiers, ×790; fig. 10, complete development of vertical walls and appearance of transverse walls below, ×790; figs. 11, 12, appearance of periclinal walls, ×790.

With the coming in of the first vertical walls in the young antheridium, several mucilage hairs arise from the cells which line the antheridial cavity. These arise near the base of the

antheridial stalk as 1-celled, slightly elongated structures with a nucleus and a highly vacuolated cytoplasm (figs. 9–13). They elongate considerably and secrete abundant mucilage around the mature antheridia.

Archegonium

The archegonia are borne on receptacles which arise as dome-like areas at the growing point of the thallus. The formation of the archegonial receptacle involves the apical cell of the thallus, so that growth for the season is checked. Several (up to 6) growing points are organized in the young receptacle from segments of the main apical cell of the thallus, and thereby a new apical cell is formed in each receptacle notch.

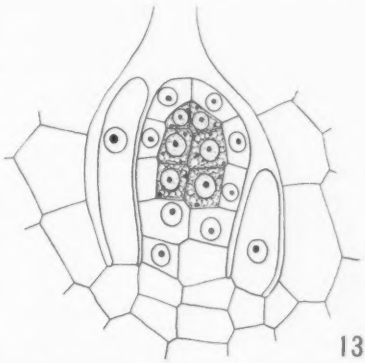
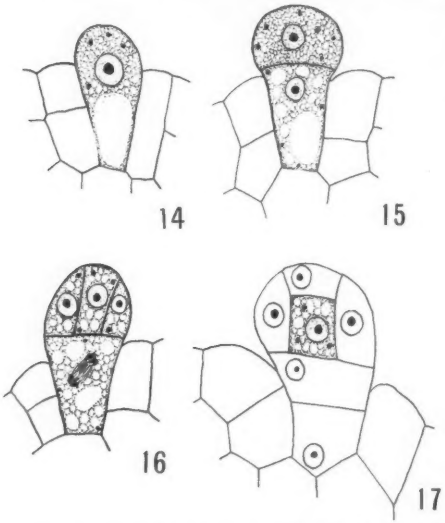


Fig. 13.—Completion of periclinal wall formation in young antheridium, ×790.

In the simpler Marchantioideae, as in *Clevea* and *Aytonia*, the main apical cell of the thallus is not involved in the formation of the archegonial receptacle, so that several successive groups of archegonia may be formed on the same thallus, or, as Miss Starr (**8**) has reported in *Aytonia*, an antheridial receptacle may follow the formation of an archegonial receptacle. Thus in these forms the archegonial receptacle represents a simple dorsal upgrowth of the thallus. In *Fimbriaria* and *Grimaldia* Leitgeb has shown that the apical cell is involved in the formation of the archegonial receptacle, and this is the situation in *Reboulia*. In the higher Marchantioideae this condition prevails, and further advance is shown merely by the greater production of archegonia over a longer period, and the formation of a long receptacle stalk earlier in the life history of the archegonia.

The archegonium initials arise from the third or fourth segment of each apical cell, and do not appear until the young receptacle is conspicuously dome-shaped. Leitgeb figures a very young

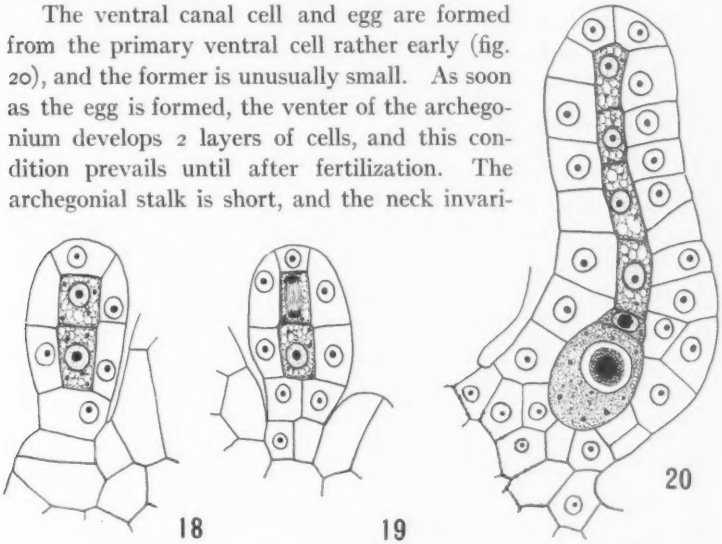
receptacle bearing an archegonium in which the cover cell has been formed, but no such condition was observed by the writer in the material used in this study. The initial arises as a superficial cell which becomes papillate, and the first transverse wall cuts off a basal cell from an outer cell (figs. 14, 15). Three vertical walls then appear in the outer cell, and the primary axial cell and primary wall cells are differentiated as in all of the Bryophytes (fig. 16). The basal cell usually divides by a transverse (figs. 16, 17) or vertical wall, but in no case was this division observed as taking place before the appearance of the 3 vertical walls. STRASBURGER (10) has reported that in *Marchantia* the outer cell undergoes a transverse division before the coming in of the 3 vertical walls, and JANCZEWSKI (5) has found a similar situation in *Preissia*. These investigators may have mistaken an early division in the basal cell for one in the outer cell, as they show no mitotic figures which would prove the case. LEITGEB figures a young archegonium of *Reboulia* in which the 3 vertical walls have followed the transverse division of the archegonium initial, and DURAND observed the same condition in *Marchantia polymorpha*. Miss STARR's figures also indicate that the early development of the archegonium of *Aytonia* is like that of *Reboulia*.



FIGS. 14–17.—Fig. 14, archegonium initial, ×790; fig. 15, appearance of transverse wall cutting off basal cell from outer cell, ×790; fig. 16, division of basal cell and differentiation of primary axial cell and primary wall cells, ×790; fig. 17, differentiation of cover cell and central cell, ×790.

Further development of the archegonium is typical (figs. 17–20). The cover cell divides by a vertical wall which accompanies the first division of the primary neck canal cell. No evidence was found to lead to the suspicion that earlier than this stage the cover cell contributes to the development of the neck cells. Numerous mitotic figures in the neck cells prove that they increase in number by intercalary divisions.

The ventral canal cell and egg are formed from the primary ventral cell rather early (fig. 20), and the former is unusually small. As soon as the egg is formed, the venter of the archegonium develops 2 layers of cells, and this condition prevails until after fertilization. The archegonial stalk is short, and the neck invari-



FIGS. 18–20.—Older archegonia: fig. 18, formation of primary neck canal cell and ventral cell from central cell, ×790; fig. 19, division of primary ventral canal cell, ×790; fig. 20, older archegonium showing formation of ventral canal cell and egg, ×790.

ably curves outward and upward (fig. 21) so as to facilitate the entrance of the sperms, as in other forms in which the archegonia are borne similarly. The mature archegonium contains 18–20 neck canal cells which break down soon after their formation. The neck in all cases shows 6 cells in cross-section, as among other Marchantiales. JANCZEWSKI states that the number of neck canal cells in *Reboulia* is 4. He probably observed a nearly mature stage like fig. 20.

In the great majority of cases only one archegonium is formed from a single segment of each apical cell, and usually the egg of each develops a sporophyte. In one case, however, 2 archegonia were observed which had developed from segments of the same apical cell. This indicates a reversion to a condition as seen in *Marchantia*, which from the standpoint of number of archegonia is very primitive. The tendency to reduce the number of archegonia among the Marchantiales reaches its highest expression in forms like *Reboulia*. CAVERS has also reported that in *Reboulia* occasionally 2 archegonia may be produced in the same receptacle notch. The apical cells of the female receptacle are checked by the formation of the archegonia and soon become lost. The lobes which develop between the archegonia grow entirely by intercalary divisions.

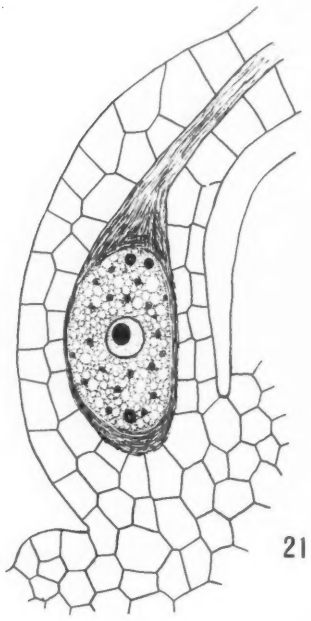


FIG. 21.—Venter of mature archegonium showing egg ready for fertilization, ×660.

Slender filaments arise below each archegonium, and grow to a considerable length. The filaments become quite numerous in each receptacle notch, and persist until the spores are shed. They are probably protective in function. The mature egg is oval and contains many plastids and several large oil globules (fig. 21). These oil drops are much larger than those found in the cells of the thallus, and they persist in the early stages of the embryo. The mature egg does not develop a cellulose wall until after fertilization.

The number of lobes developed by the mature female receptacle corresponds with the number of growing points organized in the young receptacle. The lobes are not conspicuous. In their paper CH. and R. DOUIN distinguish very carefully between the

use of the terms "rays" and "lobes" as applied to the female receptacle of the Marchantiales, as follows:

> Nous appellerons donc les rayons les divisions du capitule qui protègent les cavités pilifères, et nous réserverons le nom de lobes aux parties du capitule, qui recouvrent et protegent les involucres.

According to their interpretation and statements, neither rays nor lobes occur in *Clevea*, *Sauteria*, and *Peltolepis;* in some species of *Marchantia* and in all of the other genera of the order lobes only occur, while in most species of *Marchantia* true rays are formed. In *Preissia* both rays and lobes occur, but the rays are reduced, and the lobes very indistinct.

Summary

1. *Reboulia* comprises a single polymorphic species, *R. hemisphaerica*, belonging to the Operculatae division of the subfamily Marchantioideae.

2. The thallus bears smooth and pegged rhizoids and 2-ranked ventral scales without appendages. The body is differentiated into a dorsal and ventral region, and grows by means of a single cuneate apical cell.

3. Air chambers are abundantly formed and develop by centripetal splittings. Secondary partitions separate the primary air chambers. No chlorophyllose filaments are formed.

4. Barrel-shaped air pores are developed on the female receptacle. Those of the thallus and male receptacle are made up of a single layer of concentric cells.

5. *Reboulia* is monoecious (autoicous). The antheridial receptacle is sessile, and several may be produced during the growing season, but the formation of the archegonial receptacle terminates apical growth of the thallus and represents a definite branch system, as among the higher members of the order.

6. The antheridia develop like those of the other Marchantiales.

7. In the development of the archegonium the 3 vertical walls follow the appearance of a transverse wall in the initial cell, and further development is typical. Eighteen to 20 neck canal cells are formed, but only 4 are present at the time of division of the ventral cell.

8. Several growing points are organized in the female receptacle from segments of the apical cell of the thallus, and each new apical cell comes to lie in a receptacle notch. Only 1 (rarely 2) archegonium is formed from the immediate segment of each apical cell.

The writer is very grateful to Dr. W. J. G. LAND for the material used in the investigation and for his encouragement and suggestions during the course of the study.

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CURRENT LITERATURE

NOTES FOR STUDENTS

Mucor and Chaetocladium.—BURGEFF[1] has given an interesting account of the relations between the parasite *Chaetocladium* and the host *Mucor*. The *Chaetocladium* investigated reacts physiologically like *C. Fresenianum*, but morphologically more like *C. Brefeldi*, although its spores are larger than in the latter species. The *Mucor* host was a variety of *Mucor mucedo* which BURGEFF describes as *Mucor mucedo dependens*.

The course of infection was followed in both fixed and living condition. Fortunately the plasma of the parasite stains more deeply than that of the host, so that host and parasite can be distinguished in sectioned material. When growing together the filaments of the *Mucor* host are attracted by those of *Chactocladium*. Filaments of the parasite apparently are not attracted by those of the host until they are within a very short distance of each other. Contact of the two kinds of filaments is a stimulus which causes a slight thickening of the tip of the *Chaetocladium* hypha, inhibits its growth in length, and finally causes the formation of a cross wall cutting off the terminal portion of the filament of the parasite, which becomes the gall cell. The adjacent walls of the host and parasite dissolve, and plasma and nuclei of the host enter the gall cell, which at once swells and branches. In the gall cell the nuclei of the host are arranged peripherally and undergo division, while those of the parasite are centrally located and apparently do not divide. The gall cell is termed heterocaryotic (or a mixochimaera), since it contains two kinds of nuclei in contrast with the homocaryotic hyphae of the host and of the parasite. It is in open communication with the host hypha, but separated from the parasite by a membrane. Some of the branches from the primary gall are heterocaryotic and some homocaryotic, containing only *Mucor* nuclei. These latter branches form secondary galls in contact with pure *Chaetocladium* filaments. Apparently gall branches never contain only *Chaetocladium* nuclei, perhaps because the latter do not divide when in association with the host plasma. Branches of *Chaetocladium* from below the gall intermingle with those of the latter, thus increasing the area of contact between the gall and parasite. Apparently it is only at contact surfaces between the heterocaryotic gall and the *Chaetocladium* hyphae that diffusion to the advantage of the parasite can take place. At any rate, after contact of *Chaetocladium*

[1] BURGEFF, H., Über den Parasitismus des *Chactocladium* und die heterocaryotische Natur der von ihm auf Mucorineen erzeugten Gallen. Zeitsch. Botanik 12:1–35. *figs. 36.* 1920.

filaments with these gall cells the parasite grows out into fruiting branches. Single nuclei enter the single-spored sporangium and later divide twice, producing the four nuclei of each spore (rarely two or more than four).

The association of the plasma of host and parasite in a mixochimaera is supposed to render the protoplasmic membranes of the host permeable to diffusion of the material necessary to the normal growth of the parasite. The galls are also supposed to serve as a means of bringing about a branching of the host hypha in places such as old sporangiophores, where the ability to branch has been lost.

In seeking for a possible origin of the "sikyotic" parasitism (from σικυα = a cupping-glass) of *Chaetocladium*, BURGEFF discusses other cases of fusions in the fungi. In the anastomoses found in *Mortierella, Syncephalis,* Ascomycetes, and Basidiomycetes, a cross wall, if formed, is produced after the fusion, as shown by KNIEP, but in this case the process is connected with the distribution of nuclei in a diploid mycelium. Failing to find an analogy with vegetative processes, BURGEFF suggests that the curious type of parasitism which he has studied may have originated by way of sexual fusions; and in support of this suggestion points out the similarities between the processes involved in conjugation in the Mucors and those in the formation of his sikyotic galls in *Chaetocladium*. The suggestion is believed to need strengthening by tests with plus and minus races of host and parasite.—A. F. BLAKESLEE.

Calcium.—SHEDD[2] has found that the procedure which has been adopted by the Association of Official Agricultural Chemists for determination of calcium in soil solution does not give accurate results, due to the occlusion of calcium on the iron and aluminum precipitate that goes through the filter. He has evolved a new method which is simpler and avoids the errors of the present methods. The following findings for the Kentucky soils are of great interest. Cultivation has caused a considerable loss of calcium from these soils. The best types of these soils have the highest calcium content, and the poorest have the lowest. "Many samples have been found to be so low in calcium that their deficiency in this constituent requires consideration as well as their low phosphorus and nitrogen supply. The application of a ton of limestone or of rock phosphate per acre to such soils frequently adds more calcium than is already present. There is no doubt that, in such cases, these materials, or even moderate applications of some commercial fertilizers, are beneficial because of the plant food (calcium) they supply in addition to other good effects they may accomplish."

NELLER,[3] working on limed and unlimed plots of the New Jersey Experiment Station, finds that the oxidizing power of the limed plots is approxi-

[2] SHEDD, O. M., A proposed method for the estimation of total calcium in soils and the significance of the element in soil fertility. Soil Science **10**:1–14. 1920.

[3] NELLER, J. R., The oxidizing power of soil from limed and unlimed plots and its relation to other factors. Soil Science **10**:29–37. 1920.

mately 40 per cent higher than the unlimed; that the oxidizing power varies inversely with its lime requirement; that nitrate accumulation and bacterial numbers were higher on the limed soils, whereas the ammonia accumulation was about the same for all of the plots; that the average crop yield for the past 10 years varies closely with the present oxidizing power of the soils; and that there is a noticeable correlation between crop yield, nitrate accumulation, and bacterial numbers, but not between crop yield and ammonia accumulation.

PARKER and TRUOG[4] find a rather close relation between the calcium and nitrogen content of plants. The contents of potassium, phosphorus, and magnesium do not bear this close relation to the nitrogen content. There are two groups of agricultural plants, those having a low calcium-nitrogen ratio and a low lime requirement, and those having a high calcium-nitrogen ratio and a higher lime requirement.—WM. CROCKER.

Arctic Caryophyllaceae.—A critical study of the morphology and ecology of the Caryophyllaceae is one of WARMING'S[5] most recent contributions to the science of ecology. He divides his report into four parts, dealing respectively with (1) morphology and vegetative propagation, (2) leaf anatomy, (3) adaptations to environment, and (4) flower biology and seed reproduction.

In the first section he recognizes and describes several growth forms, illustrating by drawings of typical plants and listing the species to be referred to each form. Numerous variations of the rosette and cushion forms are distinguished, and multiplication by buds, offshoots, runners, and layers is carefully discussed. The details of the leaf structure are to be obtained from the drawings, the most important generalization being the usual absence of xeromorphic features. Palisade tissue is poorly differentiated, the mesophyll has abundant large intercellular spaces, stomata usually occur on both surfaces, and the epidermis is thin-walled and but slightly cutinized, the leaves thus resembling those of hydrophytes or shade plants. In this respect they form a striking contrast with the xeromorphic leaves of the woody evergreens of the same regions.

Among the most conspicuous features of the flower biology is the common occurrence of both protandry and polygamy, the latter being accompanied by varying degrees of reduction of stamens in the ovulate flowers. Very frequently the corolla is decidedly smaller in the ovulate flowers.—GEO. D. FULLER.

Awn and barley yield.—HARLAN and ANTHONY[6] have found that early removal of the awns of barley greatly reduces the volume and dry matter of

[4] PARKER, F. W., and TRUOG, E., The relation between the calcium and the nitrogen content of plants and the function of calcium. Soil Science **10**:49–56. 1920.

[5] WARMING, ENG., The structure and biology of Arctic flowering plants. 13. Caryophyllaceae. Meddelelser om Grönland **37**:228–342. *figs. 44.* 1920.

[6] HARLAN, H. V., and ANTHONY, S., Development of barley kernels in normal and clipped spikes and the limitations of awnless and hooded varieties. Jour. Agric. Research **19**:431–472. 1920.

the kernels at maturity. The effect is not due to shock injury, for it does not manifest itself until at least a week after removal. The rachis of the clipped spikes contains about 25 per cent more ash than the unclipped. This is probably due to the fact that the awn when present is a great ash storage organ. The high ash content of the rachis probably accounts for the marked shattering in the clipped heads. The authors say: "Hooded and awnless barleys generally yield less and shatter more than awned varieties, and there seem to be physiological reasons for this fact." It may be possible to produce non-shattering hooded and awnless sorts by using parents which normally have a low percentage of ash in the rachises. It may also be possible to obtain strains that will give good yields under arid conditions. Under humid conditions it is likely that the objections to the awns are more easily met by the use of strains with smooth awns, which, so far as known at present, have no physiological limitations.—WM. CROCKER.

A subterranean algal flora.—MOORE and KARRER[7] have demonstrated the existence of a subterranean algal flora, independent of the terrestrial flora and to a great degree of the character and locality of the soil. The investigation included an analysis of a variety of soils collected in Missouri, California, and Massachusetts. The samples were collected at different depths under sterile conditions and in localities where the soil had not been disturbed for a number of years. These were placed in bottles containing an amount of sterile algal nutrient solution and sterile sand. The growth was examined at the end of several weeks, and in this manner the algae which occurred in small amounts could easily be studied. From these investigations it was shown that algae exist in the soil to a depth of 1 m. at least under conditions which preclude the possibility of surface infection. A wide variety of species was not found, but of particular interest is the fact that *Protoderma viride* (Kützing) occurred at all depths and in all the samples obtained in the widely separated localities.—JOANNE KARRER.

Odor constituents of apples.—POWER and CHESTNUT[8] have found that the odor constituents of apples consist essentially of amylesters of formic, acetic, and capsoic acids, with a very small amount of caprylic ester and a considerable proportion of acetaldehyde. The acids mentioned are probably present also in the free state. These essential oils constitute only about 0.0007–0.0013 of 1 per cent of the weight of the entire ripe fruit. "Although amyl valerate is generally designated in chemical literature as 'apple oil,' it is quite certain that this compound has never been identified as a constituent of apples." The difference in odor of various apples is due to the difference in proportions of the oils mentioned.—WM. CROCKER.

[7] MOORE, G. T., and KARRER, JOANNE L., A subterranean algal flora. Ann. Mo. Bot. Gard. **6**:281–307. 1919.

[8] POWER, F. B., and CHESTNUT, V. K., The odorous constituents of apples; emanation of acetaldehyde from the ripe fruit. Jour. Amer. Chem. Soc. **42**:1509–1526. 1920.

Ferns of Papua.—BRAUSE[9] has published a list of Papuan ferns collected by LEDERMANN in the expedition of 1912–1913, in connection with a study of the Papuan flora by LAUTERBACH. It illustrates how any investigation of the tropics increases very materially the number of known ferns. The present list includes 555 species, distributed among 43 genera. The following 9 genera include 400 of the species: *Dryopteris* (112), *Asplenium* (52), *Trichomanes* (51), *Hymenophyllum* (35), *Alsophila* (34), *Lindsaya* (31), *Diplazium* (31), *Aspidium* (29), *Cyathea* (25). There are described 78 new species, *Dryopteris* including 24, *Alsophila* 13, *Cyathea* 7, and *Blechnum* 7, the remaining 27 new species being distributed among 12 genera.—J. M. C.

Gentes Herbarum.—Under this title BAILEY[10] has begun a new serial publication, the first fascicle containing an extensive list of plants which he collected in China in the spring and summer of 1917. The several localities are in central China, and the cultivated plants are not neglected. The collection includes 20 new species distributed among 13 genera, and 15 new varieties and forms. There are also transfers and new combinations. "The total systematic novelties and taxonomic changes are 44." The report contains also some very attractive photographs of topography and "interesting trees."—J. M. C.

Seedling anatomy.—HOLDEN,[11] in continuing studies of the anatomy of teratological seedlings, has investigated atypical seedlings of *Impatiens Roylei*, an Indian species naturalized in England. One of the two groups of these seedlings shows a very complete series illustrating the development of a "closely syncotylous condition" from the normal; while the other group shows a single cotyledon with no "macroscopic evidence" of syncotylous origin. The relation of the facts to the origin of monocotyledony is evident, but a number of alternative conclusions are still in evidence.—J. M. C.

Apogamy in Osmunda.—Mrs. BROWN[12] has succeeded in securing apogamous outgrowths in cultures of *Osmunda cinnamomea* and *O. Claytoniana*. It is stated that the only reported case of apogamy in this genus is given by LEITGEB, presumably using *O. regalis*. His observations have never been confirmed, although investigators since have tried to induce apogamy in this species under varied cultural conditions. Mrs. BROWN included *O. regalis*

[9] BRAUSE, G., Beiträge zur Flora von Papuasien. VII. Bot. Jahrb. **56**:31–160. 1920.

[10] BAILEY, L. H., Gentes Herbarum. I. A collection of plants in China. **1**:1–49. *figs. 17*. 1920.

[11] HOLDEN, H. S., Observations on the anatomy of teratological seedlings. III. On the anatomy of some atypical seedlings of *Impatiens Roylei* Walp. Ann. Botany **34**:321–344. *figs. 113*. 1920.

[12] BROWN, ELIZABETH DOROTHY WUIST, Apogamy in *Osmunda cinnamomea* and *O. Claytoniana*. Bull Torr. Bot. Club **47**:339–345. *figs.* 7. 1920.

in her cultures and obtained "apogamous outgrowths" in that species also.—J. M. C.

Peat soils.—In a discussion of the agricultural possibilities of the vast peat areas of Minnesota, estimated at 7,000,000 acres, ALWAY[13] has shown the close relationship between agricultural and ecological problems. There is a general discussion of peat soils, a synopsis of the history of peat-land control in Europe, and a review of the literature. The two systems of control discussed are those by chemical treatment and those by burning. Toxic substances in the peat and in the substratum are also considered.—GEO. D. FULLER.

Ultra-violet light and yeast.—FEUER and TANNER[14] have studied the effect of ultra-violet light on 30 different species, strains, and varieties of yeastlike fungi, and conclude that these organisms are not very resistant to ultra-violet light, and that this might be used in controlling developing yeast in the industries. Further quantitative work is under way.—WM. CROCKER.

A non-absorbing atmometer mounting.—LIVINGSTON and THONE[15] have devised a new and much simplified mounting for porous cup atmometers which prevents absorption during periods of precipitation. The necessary valve is constructed in a simple straight glass tube by the use of a piece of mineral wool and a drop of mercury.—GEO. D. FULLER.

Internal stomata.—BERGMAN,[16] having observed stomata in the endocarp of the cultivated cranberry, extended his observations to numerous ericads, finding internal stomata in a number of them. Experiments indicated that they had not retained their ability to function, and the general conclusion is advanced that they are relics retained by a "modified leaf."—J. M. C.

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[13] ALWAY, F. J., Agricultural value and reclamation of Minnesota peat soils. Univ. Minn. Agric. Exper. Sta. Bull. 188. pp. 136. *figs.* 54. 1920.

[14] FEUER, B., and TANNER, F. R., The action of ultra-violet light on the yeast-like fungi. Jour. Ind. Eng. Chem. **12**:740, 741. 1920.

[15] LIVINGSTON, B. E., and THONE, FRANK, A simplified non-absorbing mounting for porous porcelain atmometers. Science N.S. **52**:85–87. 1920.

[16] BERGMAN, H. F., Internal stomata in ericaceous and other unrelated fruits. Bull. Torr. Bot. Club **47**:213–221. *figs.* 9. 1920.

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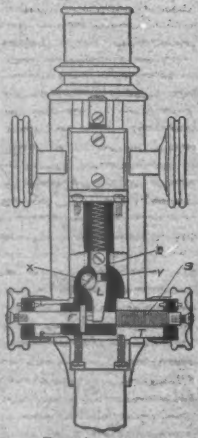

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